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THE LOSS OF HEMOLYTIC CAPACITY BY A FRACTION OF A CULTURE OF A HEMOLYTIC STREPTOCOCCUS WITHOUT CHANGE IN AGGLUTINATION CHARACTERISTICS.

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(Received for publication, February 14, 1922.)

The partial or complete loss by the hemolytic streptococcus of its ability to hemolyze red blood cells has been recorded. In the studies[1–4] on variations of streptococci as regards hemolysis and other characteristics, no complete and adequately controlled observations on the agglutination, agglutinin-binding, and agglutinogenic characteristics have been made, as far as we know, to determine whether variation in function has been associated with change in the antigenic constituents of the cocci. We are reporting, therefore, comparative observations on a strain of hemolytic streptococcus which has developed a non-hemolytic fraction.

The strain under consideration is one of the Dochez, Avery, and Lancefield[5] type strains, No. 32, which had been isolated from the lung at autopsy (bronchopneumonia following measles). This strain was obtained by us during the latter part of 1919 and has been maintained on blood-streaked agar. It was used for the production of immune serum, and also as a control strain in comparative observations on hemolysis. Thus, for more than a year it was plated on pour and

[1] A complete résumé of variations among streptococcus is given by Brown, J. H., The use of blood agar for the study of streptococci, Monograph of The Rockefeller Institute for Medical Research, No. 9, New York, 1919.

[2] Aschner, P. W., *J. Infect. Dis.*, 1917, xxi, 409.

[3] Clawson, B. J., *J. Infect. Dis.*, 1920, xxvii, 368.

[4] Rosenow, E. C., *J. Infect. Dis.*, 1920, xxvi, 597.

[5] Dochez, A. R., Avery, T. O., and Lancefield, R. C., *J. Exp. Med.*, 1919, xxx, 179.

streak blood agar plates, at frequent but irregular intervals. During this period no change was observed. When plated in April, 1921, however, it was found that 10 per cent of the colonies showed no evidence of hemolysis but resembled closely a pneumococcus colony. Isolations from both the hemolytic and green types of colonies were made and replated for purity; three substrains of each variety, which for convenience are designated H.1, H.2, H.3 and G.1, G.2, G.3, were used for study.

Up to November 1, 1921, Substrains H.1 and G.1 have been plated thirty times, the others ten times. In no instance has any change been noted in the colony characteristics on blood agar. Since the cleavage was noted, the substrains have bred true for more than 50 transfers.

Cultural Characteristics.

Described according to the recommendations of Smith and Brown[6] and Brown,[1] the hemolytic fraction has all the characteristics of the beta types with a 4 to 5 mm. transparent zone of hemolysis. The green fraction is an alpha type with no visible clearing except for the late development of a very narrow green translucent zone, which microscopically shows scarcely any diminution in the number of red cells. Immediately surrounding the colony there is no reduction of the number of cells. The surface colonies of the green type fraction are moist, green-zoned, and resemble closely a typical pneumococcus colony.

The hemolytic properties of the two fractions were also tested with the blood tube method (Lyall[7]). With the beta type, hemolysis was prompt; 0.3 cc. of a 15 hour broth culture caused complete hemolysis, and as little as 0.05 cc. caused partial hemolysis. The green fraction showed no hemolysis with 0.5 cc.; the color of the red cells was changed to a deep magenta, but not to the typical brown shown by the pneumococcus used as a control.

Morphologically and culturally the two fractions show no differences, other than those noted above. Planted in 1 per cent carbo-

[6] Smith, T., and Brown, J. H., *J. Med. Research*, 1914–15, xxxi, 455.
[7] Lyall, H. W., *J. Med. Research*, 1914, xxx, 515.

hydrate media (glucose, lactose, salicin, mannitol, raffinose, and inulin) all fishings have shown the same end-reaction. This was tested by the colorimetric method to determine the final H ion concentration. Neither fraction is soluble in bile.

Agglutination and Agglutinin Absorption Tests.

Agglutination tests were set up with the serum of a sheep immunized against the original Strain 32. Fortunately all fishings of the hemolytic and green fractions grew with a diffuse cloud in beef broth so there was no difficulty in obtaining satisfactory agglutination results. The results of the test with the sheep serum showed that both fractions were agglutinated equally well, and to the titer of the serum strain, No. 32. Likewise the absorption of this serum by either fraction removed completely the agglutinins for both fractions and for the serum strain, No. 32.

For more conclusive evidence rabbits were immunized with a representative of each of the fractions. At each injection control plates were made to check the character of the injected cocci. The serums obtained were used for agglutination and agglutinin absorption tests. The results are given in Table I.

Direct agglutination and agglutinin absorption tests, with the antiserums for both the hemolytic and green fractions, were also carried out on the original Strain 32, and on the other fishings, H.2, H.3 and G.2, G. 3, isolated at the time the cleavage was noted. The reactions of these strains were identical with those of the serum strains H. 1 and G. 1 given in Table I.

The ability of either fraction to absorb the agglutinins from the antiserum of the other fraction shows that the loss of hemolytic power has not been accompanied by any change in the agglutinogenic complex of the cocci.

Virulence Tests.

The virulence of the two strains was tested by mouse inoculation with 18 hour broth cultures. With the hemolytic variety 0.5 cc. killed uniformly, 0.25 cc. irregularly. The green type was less virulent, 1 to 1.5 cc. causing death, but only irregularly.

TABLE I.

Agglutination and Agglutinin Absorption Tests on the Hemolytic and Non-Hemolytic Fractions of Strain 32.

Antiserum.	Absorbed by substrain.	Absorbing dose.	Agglutination.						
			Substrain.	1:50	1:100	1:400*	1:2,000*	1:5,000	Broth.
H. 1†	0‡	0‡	H. 1†	++	++	++	++	+	−
			G. 1§	++	++	++	++	±	−
	H. 1	1:20‖	H. 1	−	−	−	−	−	−
			G. 1	−	−	−	−	−	−
	G. 1	1:20‖	H. 1	−	−	−	−	−	−
			G. 1	−	−	−	−	−	−
	H. 1	1:50‖	H. 1	+	×	−	−	−	−
			G. 1	±	×	−	−	−	−
	G. 1	1:50‖	H. 1	+	×	−	−	−	−
			G. 1	±	×	−	−	−	−
G. 1	0‡	0‡	H. 1	++	++	++	++	+1	−
			G. 1	++	++	++	++	+	−
	H. 1	1:20‖	H. 1	−	−	−	−	−	−
			G. 1	−	−	−	−	−	−
	G. 1	1:20‖	H. 1	−	−	−	−	−	−
			G. 1	−	−	−	−	−	−
	H. 1	1:50‖	H. 1	+	±	−	−	−	−
			G. 1	±	×	−	−	−	−
	G. 1	1:50‖	H. 1	+	±	−	−	−	−
			G. 1	±	±	−	−	−	−

Normal serums of the rabbits obtained before immunization did not agglutinate any of the strains.

Heterologous cultures of hemolytic and non-hemolytic streptococci were used as negative absorption controls. In a dose of 1:10 they did not reduce the specific titer of the serums.

++ indicates complete agglutination; +1, +, ±, ×, decreasing amount; −, no agglutination.

* To save space intermediate dilutions set up with unabsorbed and absorbed serums are not listed.

† H. 1 indicates hemolytic fraction.

‡ 0 indicates that the serum was not absorbed.

§ G. 1 indicates non-hemolytic or green fraction.

‖ Absorbing dose 1:20 or 1:50 means that the mass of cocci is to the volume of serum (diluted 1:15) as 1 is to 20 or 1 is to 50.

Attempts were made to increase the virulence of the green variant by mouse passage in the hope that the hemolytic capacity might return with the increase in virulence. After fourteen consecutive passages the virulence was raised so that $\frac{1}{60}$ cc. caused death. This increased virulence was not associated with the development of hemolytic colonies. The mouse passage caused no marked fall in agglutinability and no change in the absorptive capacity of the culture. A further attempt to restore the hemolytic capacity to the green variant was made by growing it in blood broth, but twenty-five successive transfers in this medium have resulted in no change. The hemolytic fraction has also bred true after twenty-five blood broth generations.

Replating of the original Strain 32 showed the persistence of the two varieties throughout August, 1921. At that time fresh isolations of the two fractions were made and found to be identical culturally and serologically with the previous isolations. Since then, two platings of the original strain have failed to show any green colonies although a large number of plates was made.

Tests with Other Strains.

Twenty other strains of hemolytic streptococcus, the majority from pathological conditions, which had been under cultivation for from 2 to 8 years, were tested in order to determine whether or not any loss of hemolysis had occurred. Frequent platings of these strains have shown no change in the hemolytic capacity, as all colonies give well marked zones. These observations are in contrast with those of Clawson[3] who found that out of 116 strains of hemolytic streptococcus under cultivation for 1 to 4 years, 54 showed one or more green colonies when plated in blood agar. He tested the constancy of substrains obtained from six cultures. His substrains showed little tendency to breed true, the green substrains in all instances showing reversions to the hemolytic type.

DISCUSSION.

Further observations on the antigenic character of bacterial strains in relation to functional change are needed for reconsideration of the question of so called bacterial mutation. The example which we are

reporting of an apparently sudden and permanent functional loss, that is of hemolytic capacity by a portion of a strain without associated antigenic change, is presented for this reason.

Variations in the physiological functions of bacteria occur with frequency. This has given rise to an impression held by some that the fundamental antigenic constituents are also readily subject to change. As a matter of fact, general experience indicates a high degree of stability of bacterial types as regards the antigenic qualities of their body substance. In the light of this experience it would seem more logical to assume the sceptical attitude that a change in the antigenic constituents is a fundamental and radical change, and, therefore, an unusual occurrence. The proof adduced for such a change, therefore, must be proportionately complete and convincing.

The proof must be complete in two ways. First, is the variant real or is it a contaminant of the original culture, or a contaminant added during transfer or by animal passage, etc.? Second, does the evidence advanced in favor of antigenic change prove it to be an actual basic change or simply show that a modification of the binding or reacting characteristics has occurred? The evidence for a basic change in the antigenic constituents must include a study of the agglutinogenic capacities, as well as of the reacting and combining capacities of both the variant and unchanged fraction.

CONCLUSIONS.

A culture of hemolytic streptococcus developed a non-hemolytic fraction which as determined by its agglutinogenic, agglutination, and agglutinin absorption characters was identical with the hemolytic fraction, the only variation being one of function, or physiological action. This functional variation has proved permanent, as long as observed, for both fractions have continued to breed true.

This study is offered as additional evidence in favor of the hypothesis that functional changes among bacteria are, at most, only very infrequently associated with changes in the antigenic matrix of bacteria.

THE ORGANOTROPIC, BACTERIOTROPIC, AND LEUCOCYTOTROPIC ACTIONS OF CERTAIN ORGANIC CHEMICALS.

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(Received for publication, March 1, 1922.)

Present knowledge of the manner by which chemicals influence the mechanism of infection and resistance is limited. Investigations in chemotherapy hitherto have been concerned primarily with certain organotropic and bacteriotropic activities,—a search for a chemical of a monotropic character. The influence of definite chemical entities both upon the factors vital to the integrity of the animal against an invading microorganism, and such life processes as are perhaps not essential to actual defense against bacterial disease, has not been thoroughly investigated. The work of Ehrlich, Morgenroth, Browning, and others has demonstrated that it is possible to find chemicals with less of organotropic than of bacteriotropic activity. Frequently, however, when such substances have been used against an experimental infection, the treated animal succumbed as though no drug had been given, or in a shorter time than the controls. In other words, the drug, in doses not fatal under normal conditions, apparently acts upon defensive complexes necessary to the animal in coping with an infection, having a greater influence upon this activity than upon the pathogenic microorganism. Just what the force or combination of forces is that maintains a state of stable resistance in the animal toward microorganisms can only be conjectured. Whatever other elements enter into this biological reaction, obviously the phagocyte has some share. Because of this, as evidenced in the increase of the activity of the leucocyte during the course of many infectious diseases, it was decided to study the influence of certain chemicals, not alone in respect to organotropism and to bacteriotropism, but as to leucocyto-

tropism as well. The work was undertaken in the hope that information might be gained which would enable a more intelligent choice of chemicals for chemotherapeutic purposes, and might lead to the finding of a delicate indicator by which to judge the relationship between chemical constitution and the activity exhibited by the body against an invading organism.

We wish to report the toxicity for mice (organotropism), the bactericidal action on *Staphylococcus aureus* (bacteriotropism), and the antiphagocytic influence (leucocytotropism), of certain members of seven groups of chemicals—triphenylmethane leuco bases, triphenylmethane dyes, acridines, safranines, phenazines, quinones, and cinchonas.

The work was done while in collaboration with Dr. Jacobs and Dr. Heidelberger in the study of chemotherapy.[1] The manner in which the chemicals here reported influence an experimental pneumococcus infection of mice will appear in a later publication.

The confusion that exists in the literature on phagocytosis seems to be due for the most part to variations in technique. Even in controlled phagocytic studies frequent anomalies appear, unavoidable when dealing with so delicate an indicator as the leucocyte. If, then, instead of considering results from one such experiment or series of experiments, we attempt to compare those from work done under widely differing conditions, definite conclusions are difficult to reach.

The findings of Hamburger,[2] Kolmer,[3] Manwaring and Ruh,[4] Grünspan,[5] and Smith [6] on the influence of quinine on phagocytosis can hardly be compared. Hamburger[7] obtained leucocytes from defibrinated horse blood and suspended them in horse serum. To this suspension he added the drug and incubated the mixture, thus affording opportunity for interaction between quinine and leucocytes

[1] Felton, L. D., and Dougherty, K. M., *J. Exp. Med.*, 1922, xxxv, 761.

[2] Hamburger, H. J., *Centr. Bakt., Ite Abt., Ref.*, 1913, lvii, 105. Hamburger, H. J., and Hekma, E., *Biochem. Z.*, 1908, ix, 512.

[3] Kolmer, J. A., Solis-Cohen, S., and Steinfield, E., *J. Infect. Dis.*, 1917, xx, 333.

[4] Manwaring, W. H., and Ruh, H. O., *J. Exp. Med.*, 1907, ix, 473.

[5] Grünspan, T., *Centr. Bakt., Ite Abt., Orig.*, 1909, xlviii, 444.

[6] Smith, H. L., *Lancet*, 1910, ii, 1342.

[7] Hamburger, H. J., and Hekma, E., *Biochem. Z.*, 1907, iii, 88.

before the charcoal was added. Kolmer,[3] on the other hand, used washed leucocytes procured from the peritoneal cavity of rabbits after an injection of aleuronat, and these came into contact with the quinine only after it had been incubated an hour with a heavy suspension of living Type I pneumococci. Thus in the former case, the quinine acted primarily on the leucocyte and secondarily on the substance to be phagocytosed, while in the latter, the primary action was on the pneumococcus. As might be expected, their results contradict each other, Hamburger[2] claiming an inhibition in phagocytic activity with a dilution of quinine 1:1,000, and Kolmer[3] claiming a stimulation with the same strength of the drug. Manwaring and Ruh,[4] using still another technique, found a 20 per cent stimulation from quinine in as low a dilution as 1:200. In this instance whole defibrinated blood was used with a streptococcus which had been suspended in 0.85 per cent NaCl and sterilized at 100° C. Grünspan[5] also places the optimum concentration for this drug at 1:200. Smith[6] gives 1:7,500 as the lowest dilution of quinine stimulating phagocytosis. He used washed human corpuscles, human serum, and living *B. coli*, sealing them in a Wright tube and incubating in an opsonizer. The fact that the chamber was sealed may have exerted an influence on the process.

Methods.

Although present knowledge relating to phagocytosis does not make it possible to employ a method which will give uniformly constant results, by testing the chemicals in groups, each group on the same day, with the same leucocytes, staphylococci, and guinea pig serum, we have endeavored to render the conditions of our experiments constant.

The following three methods for determining the degree of phagocytosis were tried.

1. 0.5 cc. of leucocytic suspension + 0.5 cc. of culture + 0.5 cc. of guinea pig serum 1:10 + 0.5 cc. of chemical were mixed in a 12 mm. tube and incubated for 15 minutes in a water bath at 37.5°C.

2. 0.5 cc. of leucocytic suspension + 0.5 cc. of chemical were mixed and incubated as above for 10 minutes. To this were then added 0.5 cc. of culture and 0.5 cc. of guinea pig serum 1:10, the mixture was shaken, and the tube was reincubated for 10 minutes in a water bath at 37.5°C.

3. 0.5 cc. of leucocytes suspended in undiluted guinea pig serum + 0.5 cc. of culture + 0.5 cc. of chemical were incubated for 15 minutes in a water bath at 37.5°C.

The last was the method finally adopted, since the simultaneous incubation of all constituents mixed together seemed more nearly to reproduce actual conditions of infection in the animal.

The leucocytes were procured from the peritoneal cavity of a guinea pig 15 hours after an intraperitoneal injection of a solution composed of 3 per cent aleuronat, 6 per cent starch, in 0.85 per cent NaCl, by washing out the exudate with sterile 0.5 per cent citrate in 0.85 per cent NaCl. The cells were washed three times in sterile citrate solution before being used. Opsonin and complement were furnished by fresh guinea pig serum and the organism employed as the indicator of leucocytic activity was an 18 hour broth culture of *Staphylococcus aureus*.

Smears were made after incubation, and the preparations stained by Cross'[8] method. The staphylococci contained in 100 cells were counted and the counts averaged. The result of each dilution of the chemical is shown in Tables I to VIII, the dilution quoted being calculated after the addition of all the components. Uniform dilutions of the different chemicals could not be employed, due to the varying solubilities of the drugs. Control counts were made from tubes containing leucocytes, serum, and organisms, but no chemical.

The toxicity given is the largest, non-fatal intraperitoneal dose in milligrams for 18 gm. mice.

The bactericidal action of the drugs was determined in whole blood with a 2 hour incubation period, by means of a technique described in a previous paper.[1]

Triphenylmethane Leuco Bases.

As a group, the triphenylmethane leuco bases studied inhibit phagocytosis (Table I), only 33 per cent of the total number of compounds showing a normal count in the highest dilution used. In analyzing this inhibition, consistent correlation with bactericidal potency and toxicity is hard to follow.

For instance, leucomalachite green (D 1) and leucobenzein (D 8) have the same strength as regards bactericidal action, killing *Staphylococcus aureus* at 1:400. But D 1, causing a 60 per cent reduction of the phagocytic index at 1:40,000, permits an approximately normal count at 1:160,000, while D 8 at 1:600,000 shows an 80 per cent inhibition of this activity. The maximum non-lethal dose for D 1

[8] Cross, H. B., *Bull. Johns Hopkins Hosp.*, 1921, xxxii, 350.

is 1.0 mg., for D 8, 2.5 mg., so that D 1, while two and one-half times as toxic and having the same bactericidal action, allows a much greater degree of phagocytosis than D 8.

p-Hydroxyleucomalachite green (D 2) and *p*-aminoleucomalachite green (D 3) show divergence in another direction. Both have a maximum non-lethal dose of 1.0 mg. D 3 exerts no bactericidal power at 1:400, while D 2 kills at that dilution. At 1:160,000, D 2 exhibits an approximately normal index, D 3 a 33 per cent reduction. At 1:320,000, D 2 shows a 50 per cent reduction and D 3 only a 25 per cent.

A third variation in effect is seen between *o*-hydroxyleucomalachite green (D 9) and *o*-methoxyleucomalachite green (D 11). The phagocytic indices are reduced 80 per cent by both chemicals at 1:320,000, but neither has any bactericidal power at 1:400, and their maximum non-lethal doses differ widely, D 9 being two and one-half times as toxic as D 11.

Triphenylmethane Dyes.

In comparing the specific dyes with their leuco bases the same irregularity is found. Neither *o*-hydroxymalachite green (D 7) nor its leuco base (D 9) in 1:400 possesses bactericidal power; D 9, however, is two and one-half times as toxic, and at 1:600,000 reduces the phagocytic index 90 per cent as compared with 25 per cent by D 7 in the same dilution (Table II).

In the case of the *p*-methoxymalachite green (D 12) and its leuco base (D 10), the dye is more toxic and bactericidal than the leuco base, but from 1:15,000 to 1:1,500,000, D 12 shows a normal phagocytic index, while at 1:600,000, D 10 reduces it 66 per cent. It may be noted here that *p*-methoxymalachite green (D 12) and ethylviolet chloride (D 32) are exceptional in allowing normal indices in a bactericidal dilution.

TABLE I.

Triphenylmethane Leuco Bases.

Chemical.	Maximum non-lethal dose intraperitoneally.		Bactericidal for staphylococci in whole blood in 2 hrs.	Effect on phagocytic index of different dilutions of chemicals.				
	Mg.	Dilution in mouse.		1:40,000	1:80,000	1:160,000	1:320,000	Control.
(D 1) Leucomalachite green.	1.0	1:3,000	0 at 1:400	3.5	3.8	8.6	11.0	9.9
(D 3) *p*-Aminoleucomalachite green.	1.0	1:3,000	∞ " 1:400	3.2	4.1	6.0	7.1	9.9
(D 6) *p*-Methylleucomalachite green.	2.5	1:1,200		4.4	4.0	7.3	4.4	9.9
				1:1,500	1:15,000	1:150,000	1:1,500,000	Control.
(D 13) *p*-Ethoxyleucomalachite green.	0.5	1:6,000	∞ at 1:400	3.6	5.0	5.0	6.2	6.0
				1:40,000	1:80,000	1:160,000	1:320,000	Control.
(D 2) *p*-Hydroxyleucomalachite green.	1.0	1:3,000	+ at 1:400	3.2	5.9	8.8	6.4	9.9
				1:600	1:6,000	1:60,000	1:600,000	Control.
(D 9) *o*-Hydroxyleucomalachite green.	0.5	1:6,000	∞ at 1:400	2.4	2.7	2.6	2.5	12.3
				1:1,500	1:15,000	1:150,000	1:1,500,000	Control.
(D 14) 2, 4-Dihydroxyleucomalachite green.	1.25	1:2,400	∞ at 1:400	2.8	3.9	6.6	4.3	6.4
				1:600	1:6,000	1:60,000	1:600,000	Control.
(D 11) *o*-Methoxyleucomalachite green.	1.25	1:2,400	∞ at 1:400	1.2	2.3	2.1	2.5	12.3

TABLE I—*Concluded.*

Chemical.	Maximum non-lethal dose intraperitoneally.		Bactericidal for staphylococci in whole blood in 2 hrs.	Effect on phagocytic index of different dilutions of chemicals.				
	Mg.	Dilution in mouse.		1:40,000	1:80,000	1:160,000	1:320,000	Control.
(D 4) Leucocrystal violet.	1.0	1:3,000	∞ at 1:350	2.4	1.2	4.4	3.4	9.9
				1:600	1:6,000	1:60,000	1:600,000	Control.
(D 10) *p*-Methoxyleucomalachite green.	2.5	1:1,200	0 at 1:400	4.5	3.2	2.4	4.2	12.3
(D 8) Leucobenzein.	2.5	1:1,200	0 " 1:400		3.2	2.6	2.0	12.3

The blanks in the tables indicate that the cells in those dilutions were too disintegrated to count.

In general, the leuco compounds are less bactericidal than the dyes, the difference being quite marked in some cases.

(D 18) Malachite green nitrate..... 1 : 6,400
(D 1) Leucomalachite green..... >1 : 400
(D 16) *p*-ethoxymalachite green chloride..... 1 : 12,800
(D 13) *p*-ethoxyleucomalachite green chloride..... >1 : 400
(D 15) *o*-methoxymalachite green nitrate..... 1 : 6,400
(D 11) *o*-methoxyleucomalachite green nitrate.....>1 : 400

This bactericidal relationship between the dye and its corresponding leuco base is not found in regard to the antiphagocytic action. Without exception both leuco bases and dyes decrease the phagocytic index, and the dilution represented by the largest non-lethal dose in the mouse is more leucocytotropic than bacteriotropic.

Acridines.

The acridines represented in Table III yield findings very similar to that with the triphenylmethane dyes, although as a group they are not so bactericidal, nor is perhaps the antiphagocytic action so great. In comparing the chemicals of this group, proflavine (D 26) stands out as possessing almost ideal characteristics, very similarly to *p*-methoxymalachite green (D 12) and ethyl violet (D 32) of the triphenylmethane dyes; the tropic relationships of the chemicals are such that a dose non-lethal for mice is also bactericidal for staphylo-

TABLE II.

Triphenylmethane Dyes.

Chemical.	Maximum non-lethal dose intraperitoneally.		Bactericidal for staphylococci in whole blood in 2 hrs.	Effect on phagocytic index of different dilutions of chemicals.				
	Mg.	Dilution in mouse.		1:600	1:6,000	1:60,000	1:600,000	Control.
(D 18) Malachite green nitrate.	0.6	1:5,000	0 at 1:6,400	0.24	—	11.5	15.0	14.1
				1:1,200	1:12,000	1:120,000	1:1,200,000	Control.
(D 16) *p*-Ethoxymalachite green chloride.	0.6	1:5,000	0 at 1:12,800	—	1.9	2.8	—	4.2
(D 19) *p*-Hydroxymalachite green chloride.	0.3	1:9,000	∞ " 1:1,600	—	—	1.8	2.3	4.0
				1:600	1:6,000	1:60,000	1:600,000	Control.
(D 7) *o*-Hydroxymalachite green.	1.25	1:2,400	∞ at 1:400	1.8	2.1	3.0	3.0	4.1
				1:1,500	1:15,000	1:150,000	1:1,500,000	Control.
(D 15) *o*-Methoxymalachite green nitrate.	0.6	1:5,000	0 at 1:6,400	4.1	4.4	6.8	5.0	6.4
				1:40,000	1:80,000	1:160,000	1:1,320,000	Control.
(D 5) Hexamethylviolet (crystal).	1.0	1:3,000	0 at 1:350 24 hrs. 0 at 1:39,400	0.0	3.0	8.8	4.4	7.4
				1:900	1:9,000	1:90,000	1:900,000	Control.
(D 20) *p*-Tolylmalachite green chloride.	0.03	1:90,000	0 at 1:2,000	—	—	—	—	9.1

TABLE II—*Concluded.*

Chemical.	Maximum non-lethal dose intraperitoneally.		Bactericidal for staphylococci in whole blood in 2 hrs.	Effect on phagocytic index of different dilutions of chemicals.				
	Mg.	Dilution in mouse.		1:1,200	1:12,000	1:120,000	1:1,200,000	Control.
(D 17) *o*-Chloromalachite green chloride.	0.6	1:5,000	0 at 1:1,600	—	0.8	3.4	2.3	3.4
				1:600	1:6,000	1:60,000	1:600,000	Control.
(D 24) *p*-Chloromalachite green nitrate + 3.5 H_2O.	0.5	1:6,000	+ at 1:2,500	—	1.3	2.2	3.2	7.1
(D 21) Brilliant green nitrate + 1 H_2O.	0.003	1:900,000	0 " 1:4,000	3.1	5.0	3.3	7.9	7.8
(D 32) Ethylviolet chloride	0.125	1:24,000	0 " 1:8,000	4.5	13.0	12.0	13.0	12.3
(D 31) 3, 4-Methylene-dihydroxymalachite green chloride + 4 H_2O.	0.06	1:50,000	0 " 1:8,000	6.8	11.0	7.0	12.5	12.3
(D 27 a) 2-Ethoxy-4′, 4″-bisdimethylaminotriphenylcarbinol.	0.03	1:90,000	0 " 1:500	—	1.0	—	9.0	14.1
(D 27 b) *o*-Ethoxymalachite green.	1.25	1:2,400	0 " 1:500	—	—	—	—	14.1
(D 22) *p*-Nitromalachite green chloride.	0.03	1:90,000	+ " 1:2,000	—	1.3	5.5	4.8	7.1
				1:1,500	1:15,000	1:150,000	1:1,500,000	Control.
(D 12) *p*-Methoxymalachite green.	0.5	1:6,000	0 at 1:3,200	4.5	8.0	7.5	8.5	7.6

coccus and in this concentration not antiphagocytic. Since the introduction of acriflavine by Ehrlich[9] members of this group of chemicals have been employed in the treatment of various infections.

Browning and Cohen[10] have recently reported on the antiseptic properties of a rather complete series of acridines, and although not all derivatives were the same

[9] Ehrlich, P., and Benda, L., *Ber. chem. Ges.*, 1913, xlvi, 1931.

[10] Browning, C. H., and Cohen, J. B., *Brit. Med. J.*, 1921, ii, 695.

TABLE III.

Acridines.

Chemical.	Maximum non-lethal dose intraperitoneally.		Bactericidal for staphylococci in whole blood in 2 hrs.	Effect on phagocytic index of different dilutions of chemicals.				
	Mg.	Dilution in mouse.		1:600	1:6,000	1:60,000	1:600,000	Control.
(D 54) 3, 6-Dihydroxy-acridine.	1.25	1:2,400	∞ at 1:1,000	0.5	5.2	7.9	15.0	10.3
(D 53) 3, 6-Diamino-9-phenylacridine.	1.25	1:2,400	∞ " 1:1,000	—	1.3	0.5	1.6	10.3
(D 60) Diamino-*n*-methyl-acridone.	1.25	1:2,400	∞ " 1:500	2.5	4.5	11.3	7.1	15.1
(D 43) 9, 10-Dimethyl-acridinium chloride.	0.625	1:5,000	∞ " 1:500	—	3.0	6.1	6.1	12.3
				1:200	1:2,000	1:20,000	1:200,000	Control.
(D 34) 3, 6-Diamino-10-methylacridinium chloride + 1 H_2O.	0.5	1:6,000	0 at 1:1,000	0.8	3.9	7.2	6.2	12.3
				1:600	1:6,000	1:60,000	1:600,000	Control.
(D 57) 2-Amino-6-hydroxy-acridine dihydrochloride.	2.5	1:1,200	∞ at 1:500	—	3.0	1.2	1.2	10.3
(D 26) Diaminoacridine sulfate + 3 H_2O.	2.5	1:1,200	0 " 1:500	1.7	17.8	14.0	13.0	14.1
(D 49) Leucocyanotrypaflavine dihydrochloride.	0.312	1:9,000	+ " 1:500	1.8	5.6	9.2	9.6	10.3
(D 42) Cyanotrypaflavine.	0.078	1:38,400	0 " 1:500	4.0	6.4	2.6	8.7	12.3
(D 56) Acridine orange dihydrobromide.	0.625	1:5,000	∞ " 1:500	—	—	1.9	3.2	10.3
(D 40) Homoflavine ("acridine yellow").	0.6	1:5,000	∞ " 1:1,000	—	4.5	5.5	7.8	12.3

as those given here, confirmation was made in respect to bactericidal action on staphylococcus. These authors did not study the influence of the group on the phagocytic index. Gay and Morrison[11] using acriflavine claim negative chemotherapeutic results in experimental streptococcus empyema in rabbits, regardless of

[11] Gay, F. P., and Morrison, L. F., *J. Infect. Dis.*, 1921, xxviii, 1.

the high bactericidal potency of the drug against this organism. They show phagocytosis to be inhibited by strong concentrations, such as have usually been employed, but state that the dye sterilizes considerable quantities of pus in the test-tube in a dose which does not inhibit phagocytosis.

Browning, Gulbransen, Kennaway, and Thornton[12] report that acriflavine kills staphylococcus in a dilution of 1:100,000 in serum and does not inhibit phagocytosis above 1:500. It is difficult to compare our results with theirs, as a different medium was used, the incubation period was shorter in performing the bactericidal test, they did not consider phagocytosis inhibited unless the inhibi-

TABLE IV.

Safranines.

Chemical.	Maximum non-lethal dose intraperitoneally.		Bactericidal for staphylococci in whole blood in 2 hrs.	Effect on phagocytic index of different dilutions of chemicals.				
	Mg.	Dilution in mouse.		1:600	1:6,000	1:60,000	1:600,000	Control.
(D 63) Methylnaphthophenazonium chloride.	0.156	1:19,200	+ at 1:500	—	—	9.7	8.6	15.1
(D 62) Isorosindulin nitrate.	2.5	1:1,200	+ " 1:500	—	2.4	—	—	15.1
(D 37) Phenosafranine chloride.	0.078	1:38,400	+ " 1:500	1.8	8.5	7.1	9.0	12.3
				1:200	1:2,000	1:20,000	1:200,000	Control.
(D 47) Phenylrosindulin chloride.	0.625	1:4,800	0 at 1:500	0.8	1.3	2.8	2.1	12.3

tion exceeded 50 per cent, and they used human materials in their method. Despite the toxic action of proflavine and acriflavine for the phagocyte, they seem to have some local therapeutic action. According to Davis[13] both of these chemicals, following either an intravenous injection or *per os* administration, render the urine bactericidal for both staphylococcus and the colon bacillus. Davis and Harrell[14] also report a therapeutic action of acriflavine in treatment of gonorrheal urethritis.

Safranines.

The small number of safranines studied does not warrant any general deductions as to this group of compounds. But for the staphylo-

[12] Browning, C. H., Gulbransen, R., Kennaway, E. L., and Thornton, L. H. D., *Brit. Med. J.*, 1917, i, 73.

[13] Davis, E. G., and Beck, G. H., *J. Urol.*, 1921, v, 215.

[14] Davis, E. G., and Harrell, B. E., *J. Urol.*, 1918, ii, 257.

coccus, the chemicals shown in Table IV exert a very low bactericidal action. And they are markedly antiphagocytic with the exception of phenosafranine, which produces a 25 per cent reduction in the phagocytic index in a dilution of 1:600,000.

TABLE V.

Phenazines.

Chemical.	Maximum non-lethal dose intraperitoneally.		Bactericidal for staphylococci in whole blood in 2 hrs.	Effect on phagocytic index of different dilutions of chemicals.				
	Mg.	Dilution in mouse.		1:600	1:6,000	1:60,000	1:600,000	Control.
(D 65) *unsym.*-Diaminophenazine hydrochloride.	1.25	1:2,400	∞ at 1:500	—	6.3	5.6	10.6	15.5
(D 38) *unsym.*-Diaminosafranine hydrochloride.	2.5	1:1,200	∞ " 1:500	—	2.8	6.5	8.9	12.3
				1:200	1:2,000	1:20,000	1:200,000	Control.
(D 46) *sym.*-Diaminophenazine hydrochloride.	1.25	1:2,400	+ at 1:500	—	2.8	2.9	4.0	12.3
				1:600	1:6,000	1:60,000	1:600,000	Control.
(D 36) Toluylene red hydrochloride.	1.25	1:2,400	∞ at 1:500	3.2	6.0	3.3	8.4	12.3
				1:200	1:2,000	1:20,000	1:200,000	Control.
(D 44) Dimethylnaphthoenrhodine hydrochloride.	0.6	1:5,000	∞ at 1:500	—	3.5	6.5	5.0	12.3

Phenazines and Quinones.

The phenazines (Table V), and quinones (Table VI) both may be classed as drugs that are not bactericidal for staphylococci, but they are antiphagocytic in dilutions non-toxic for a mouse, with the exception of sodium chloranilate in the quinone group. This compound apparently has no bactericidal action, yet permits of phagocytosis within 25 per cent of normal in a dilution of 1:600.

TABLE VI.

Quinones.

Chemical.	Maximum non-lethal dose intraperitoneally.		Bactericidal for staphylococci in whole blood in 2 hrs.	Effect on phagocytic index of different dilutions of chemicals.				
	Mg.	Dilution in mouse.		1:600	1:6,000	1:60,000	1:600,000	Control.
(D 58) Quinone.	0.625	1:5,000	∞ at 1:1,000	4.0	2.3	1.0	4.2	10.3
(D 59) *p*-Nitrosophenol.	0.312	1:9,000	+ " 1:50	2.3	3.2	5.6	13.0	15.5
(D 64) Sodium chloranilate.	5.0	1:600	∞ " 1:500	11.1	14.2	14.2	2.0	15.1
(D 67) Anilino-*β*-naphtha quinone.	1.25	1:2,400	∞ " 1:1,000	—	6.0	12.0	7.7	10.3

Cinchonas.

The cinchona compounds in Table VII are the members of a group of alkaloids on which we reported in a previous paper.[1] It was found that the aromatic compounds had the power of killing, rapidly, multiple lethal doses of virulent pneumococci when organisms and drugs were injected simultaneously into the peritoneal cavity of mice. In this respect, all cinchona derivatives were found to be superior to optochin, but the different aromatic substitution products were possessed of varying degrees of bactericidal action *in vitro* and *in vivo*. Intravenous treatment with these drugs lowered the resistance of the mice as did any method of treatment except *per os*, when given at a site other than the one at which the organisms had been injected. Although mice with definitely established pneumococcus infection were not cured, these compounds, under specified conditions, had a measurable amount of protective action, greater perhaps than that of any drug used heretofore against a bacterial infection.

At a glance the compounds in therapeutic doses in Table VII are seen to possess marked antiphagocytic activity, and with the possible exception of C 29, the aromatic compounds are seen to be more leucocytotropic than optochin. The question naturally arises, whether this antiphagocytic property is the cause of the therapeutic failure of the chemicals in an established infection.

TABLE VII.

Cinchonas.

Chemical.	Maximum non-lethal dose intraperitoneally.		Bactericidal for staphylococci in whole blood in 2 hrs.	Effect on phagocytic index of different dilutions of chemicals.			
	Mg.	Dilution in mouse.		1:1,000	1:2,000	1:4,000	Control.
(C 29) Hydroquinine chloroacetylanilide.	0.5	1:6,000		—	6.0	3.7	7.7
(C 36) Dihydroquinine *p*-chloroacetylaminophenol hydrochloride.	2.5	1:1,200	+ at 1:1,000	—	—	2.4	7.7
(C 40) Dihydroquinine *m*-chloroacetylaminophenol hydrochloride.	1.5	1:1,900	+ " 1:2,000	1.9	3.2	∞	7.7
(C 110) Dihydroquinine 4-chloroacetylaminopyrocatechol hydrochloride.	4.0	1:750	+ " 1:500	—	1.2	0.72	7.7
(C 11) Optochin (ethylhydrocupreine).	3.0	1:960	+ " 1:500	5.2	1.8	3.36	7.7
(C 9) Hydroquinine.	1.0	1:3,000		4.6	6.0	4.0	7.7

Bordet's experiments[15] showing that the resistance of an animal is lowered after it has been partially depleted of leucocytes by means of carmine, and also Lippman's work[16] proving that optochin has no protective action in animals whose leucocyte count has been lowered by thorium, may be cited in support of the postulate that a drug which inhibits the action of the leucocytes will have little value in treatment of a bacterial disease. However, the experiments of neither Bordet[15] nor Lippman[16] are conclusive for the reason that the chemicals used to decrease the number of white cells may have exerted a toxic action on other functions vital to the resistance of the animal. Acton[17] has demonstrated that a number of cinchona derivatives inhibit completely the migration of leucocytes in a dilution of 1:500. The classical work of Binz[18] shows that quinine itself, along with its other general protoplasmic toxicities, inhibits phagocytosis.

[15] Bordet, J., Studies in immunity, translated by Gay, F. P., New York, 1909, 30.

[16] Lippman, *Z. Immunitätsforsch., Orig.*, 1915–16, xxiv, 107.

[17] Acton, H. W., *Lancet*, 1922, i, 124.

[18] Binz, C., Das Chinin. Nach den neuern pharmakologischen Arbeiten dargestellt, Berlin, 1875.

With the four cinchona derivatives, as with optochin, we have been able to show that migration of leucocytes follows almost immediately after the injection of a non-toxic dose of the drug into the peritoneal cavity of a mouse. The experiments reported in Table VIII were carried out by injecting 0.5 mg. of drug in a 1 cc. volume into the peritoneum of mice. At 1, 2, 3, 4, and 24 hours respectively, a small quantity of fluid was removed, diluted with a known amount of Turk's solution, shaken, and counted in the usual manner. Obviously, the drugs have a positive chemotactic influence on leucocytes in mice, since these migrate to the site of injection. Inasmuch as the cinchona compounds, as has been shown above, inhibit the phagocytosis of staphylococcus and are also proven to have positive chemotactic characteristics, it would seem that the antiphagocytic action is due, to a large extent, to the paralysis of the function of the leucocytes and not to destruction of the cells.

TABLE VIII.

Chemotactic Influence of Aromatic Cinchona Derivatives.

Chemical.	Duration of observation.				
	1 hr.	2 hrs.	3 hrs.	4 hrs.	24 hrs.
(C 29) Hydroquinine chloroacetylanilide.	2,110*	2,340	5,740	4,000	9,400
(C 36) Dihydroquinine *p*-chloroacetylamino-phenol hydrochloride.	3,520	5,360	5,140	4,300	No fluid.
(C 40) Dihydroquinine *m*-chloroacetylamino-phenol hydrochloride.	2,720	3,020	4,620	5,000	" "
(C 110) Dihydroquinine 4-chloroacetylamino-pyrocatechol hydrochloride.	3,860	4,200	6,800	9,840	63,680
(C 11) Optochin (ethylhydrocupreine).	740	1,660	22,300	5,360	No fluid.

* The numbers refer to cells per c.mm. of fluid.

DISCUSSION AND SUMMARY.

We are dealing, as the results show, with groups of chemicals, all of which, whether bacteriotropic or not, greatly inhibit the engulfing of *Staphylococcus aureus* by leucocytes. Not a sufficiently large number of experiments was performed in attempt to cure experimental staphylococcus infections to warrant any conclusion in regard to possible therapeutic activity against this organism. How-

ever, as will appear in another paper, the only group out of the seven which definitely possessed an *in vivo* bactericidal action against pneumococcus is that of the cinchona derivatives. Certain members of the other chemical groups studied, although bactericidal in a very high dilution,—chemicals in which the concentration of a non-lethal dose was many times greater than that required to kill multiple minimal lethal doses of organisms *in vitro*,—had no certain effect when bacteria and drug were injected simultaneously into the peritoneal cavity of a mouse. In fact, the treated mouse often died before the controls.

If we may assume,—leaving out of consideration the practical significance of *in vivo* chemical destruction and excretion following the injection of the drug into the animal,—that the failure of these chemicals to exhibit a benign influence on a systemic infection in cases in which the drug can be used in a bactericidal dilution, is due to their antiphagocytic property, only one step has been taken in analysis of the factors vital for the defense of the animal against a specific microorganism. Why do these chemicals inhibit leucocytic activity? Is it because of their influence upon complement, opsonin, or the leucocyte itself, or some special one function that determines the ability to ingest bacteria? Only further work can definitely settle this question and also determine whether or not such an analysis would be of practical importance in a rational development of chemotherapy.

The ideal chemotherapeutic agent may be one that has an *in vivo* bactericidal potency and a negligible or stimulatory phagocytic action in doses non-lethal for the experimental animal. However difficult such a drug may be to find, it seems unlikely that the ultimate success in chemotherapy will be so simple. Again, it is conceivable that a secondary action of a drug, although leucocytotropic and not bacteriotropic, may bring about conditions in the animal body that will enable it to throw off the invading organism. Or finally, a drug compatible with the forces necessary to the host's defense and possessing *in vivo* bactericidal action to a greater or less degree may be the chemical sought for, the goal toward which we should strive, to achieve a rational chemotherapy for infectious diseases.

CONCLUSIONS.

With certain members of the triphenylmethane dyes and leuco bases, safranines, phenazines, quinones, and cinchona groups of chemicals, there exists no consistent parallelism between the bacteriotropic activities and the organotropic and leucocytotropic activities.

All the chemicals tested possess a leucocytotropic action, as measured by the decreased ability of leucocytes to ingest staphylococci. This action against the functional activity of the leucocyte is more pronounced than the organotropism or bacteriotropism (for staphylococcus).

Four aromatic cinchona compounds, hydroquinine chloroacetylanilide hydrochloride (C 29), dihydroquinine *p*-chloroacetylaminophenol hydrochloride (C 36), dihydroquinine *m*-chloroacetylaminophenol hydrochloride (C 40), dihydroquinine 4-chloroacetylaminopyrocatechol hydrochloride (C 110), and optochin (C 11) are markedly antiphagocytic in their therapeutic dose. They possess a positive chemotactic action for leucocytes when injected into the peritoneal cavity of mice.

In the cases of *p*-methoxymalachite green (D 12), ethyl violet chloride (D 32), and diaminoacridine sulfate (D 26) the condition was approached in which the concentration of a non-lethal dose for mice is staphylotropic and not leucocytotropic.

THE SIGNIFICANCE OF COLOSTRUM TO THE NEW-BORN CALF.

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The high mortality of calves during the 1st week of life has been the subject of researches for a number of decades. The chief symptoms preceding death are diarrhea, whence the name scours, inflammation of the umbilical stump (omphalitis), and multiple exudative arthritis. The concensus of opinion today is that there is a more or less close relation among these clinical manifestations and that, as pointed out by C. O. Jensen,[1] the chief infecting agents are races of *Bacillus coli*. In the latest summaries of handbooks covering this subject the emphasis is placed upon the infectious agents and all the measures that are recommended involve isolation, cleanliness, disinfection, and specific serum treatment.

Running parallel with these investigations but hardly influenced by them are studies on the transmission of immune bodies from mother to fetus and nursling. The fundamental studies of Ehrlich on ricin and abrin immunity transmitted to mice in the milk of immune mothers were followed by a series of investigations involving both normal and artificially induced antibodies in the mother's milk. These showed that not all species act alike, that some may transmit antibodies to the fetus *in utero*, others only in the milk.

The difficulty experienced in keeping calves alive which had not received colostrum from the mother led to a more thorough study of the effect of withholding this first milk. The significance of colostrum to the new-born calf is not set forth in medical or veterinary

[1] Jensen, C. O., in Kolle, W., and von Wassermann, A., Handbuch der pathogenen Mikroorganismen, Jena, 2nd edition, 1913, vi, 121.

literature with any definiteness. This fluid is generally assumed by some as necessary to cause evacuation of the fetal fluids and solids from stomachs and intestines. Others think that a cathartic like castor oil may replace it. In formulating measures for preventing calf scours Hutyra and Marek,[2] probably basing themselves on the work of C. O. Jensen[3] who stated in 1905 that calves die of enteritis if fed cooked or pasteurized milk during the first 24 hours of life, advise feeding the dam's colostrum for a day before heated milk is fed. They suggest that the fore milk be rejected since it might be infected. In the carrying out of the Bang system for the control of bovine tuberculosis, some have withheld colostrum, others have allowed the calf to suckle the dam immediately after birth, although the original method permits the calf to remain with the dam until it has taken the first milk.

Considerable work on the problem of feeding new-born calves has been done by W. L. Williams and associates.[4] Although the factor of colostrum entered, it was complicated with the use of scours sera and the boiling or pasteurizing of milk. No definite experiments on the simple exclusion of colostrum from the diet and the association with these of adequate controls by which the unknown, uncontrolled factors might be balanced are reported.

J. Traum[5] takes up the question of raising calves from a tuberculous herd by excluding the colostrum and milk of the reacting dam. Traum does not, however, state how many calves did not receive colostrum or milk immediately following the colostric period, for he writes that in the herd in which the experiment was going on "there were usually about 25 fresh cows yielding sufficient colostrum to frequently supply the calves born in reacting herd." Without doubt Traum did raise calves without colostrum, but he does not state the number as compared with those that did receive it nor give any subsequent history of the calves, nor does he report any losses by the way.

So far as it has been possible to examine the literature, the statement is warranted that no experiments dealing exclusively with the

[2] Hutyra, F., and Marek, J., Spezielle Pathologie und Therapie der Haustiere, Jena, 4th edition, 1920, i, 163.

[3] Jensen, C. O., *Z. Thiermed.*, 1905, ix, 121.

[4] Williams, W. L., et al., *Rep. New York State Vet. College*, 1914–20.

[5] Traum, J., *J. Am. Vet. Med. Assn.*, 1921, lix, 755.

withholding of colostrum have been made which at the same time include a number of controls sufficient to eliminate highly virulent epizootics of scours. For this reason it has seemed justified to record the following experiments with a certain number of details concerning the individual calves gathered together in an appendix.

EXPERIMENTAL WORK.

In order to balance as far as possible the many unknown factors entering into the undertaking, two experiments were carried on simultaneously. One group of calves was to have colostrum, the other not. The calves were obtained from the same large herd. To make sure that none that had suckled the mother soon after birth should be included in the group not having taken colostrum, only those born during the day and definitely known to the attendant to be without food were chosen. These were removed with as little exposure as possible to the Department units, a distance of $1\frac{1}{2}$ miles. The calf was covered with a blanket, placed in a crate, and transported in a protected autotruck. The units were artificially heated. In the early experiments the calves were gently rubbed with cloths until nearly dry. Later they were washed with hot water containing some soda to soften the mucus adhering to the hair and thoroughly rubbed until dry. The umbilicus in later cases was snipped off if longer than 1 inch, gently manipulated to remove fluids, dipped in 0.1 per cent bichloride of mercury, and dusted with boric acid powder.[6] In some the bichloride was omitted. The milk was fed soon after milking and was raw in all cases.

The group which took colostrum comprised ten calves. All of these survived the danger period and were kept various periods of time thereafter. Three died unexpectedly after 25, 38, and 45 days respectively (Nos. 757, 694, and 715). The protocols indicate that death was most probably due to some kind of poison and experiments are under way to clear up if possible the cause of death. Whatever this cause may have been, the evidence is quite conclusive that there was no ordinary infection involved, since in the two from whose organs cultures were made bacteria could not be demonstrated even though in one case (No. 715), death having occurred early Sunday

[6] Williams, W. L., *Rep. New York State Vet. College*, 1913–14, 163.

morning, the autopsy was not made until after 28 hours refrigeration. A condition common to the three animals was the presence of punctiform hemorrhages throughout the intrathoracic portion of the thymus.

Of the second group of twelve calves which received no colostrum, nine died and three survived. These may be grouped according to the length of life. Seven of the calves died within 6 days. Thus four (Nos. 663, 685, 699, and 566) died within 3 days, one (No. 895) in $3\frac{1}{2}$ days, one (No. 894) in $4\frac{1}{2}$ days, and one (No. 665) in $5\frac{1}{2}$ days. One (No. 682) not included in the figures was too weak to take food and died within 2 days.

Before considering the gross appearances presented at autopsy, it should be stated that in new-born calves minute hemorrhages are almost regularly encountered in certain organs. They are rarely absent from the auriculoventricular valves of the heart. These have been made the subject of special study by Laura Florence.[7] The mucosa of the leafy or expanded portion of the fourth stomach is regularly sprinkled over with petechiæ. The area involved varies from a few centimeters to the entire stomach. In some cases the hemorrhages are deep, in others superficial. Each spot is then capped with a bit of digested blood. Notably the pyloric valve is the seat of minute hemorrhages and in calves several months old shallow ulcers are occasionally still present on this valve. Less frequently the lowest 3 to 4 inches of the rectal mucosa is deeply congested and blood is now and then seen on the discharges. The ileocecal valve in rare instances is permeated with hemorrhages. In a few cases the mucosa of the rumen is involved in hemorrhages. If we except the heart valves, the hemorrhages, so far as studied, may be tentatively considered as mechanical in origin and due to conditions arising during parturition. Wherever in the present paper these hemorrhages are mentioned, the above facts should be borne in mind.

The appearances at autopsy differed somewhat from animal to animal and were due to differences in the blood content of the organs. The mucosa of the small intestine was in some instances pale, in others deeply congested in parts or throughout. The large intestine also varied in this respect. In all cases the small intestine was more deeply congested than the large. The other organs most involved were the kidneys. These varied from a nearly normal coloration and consistency to an intense congestion of the entire organ and a much firmer consistency. The urine taken from the bladder was free from blood or hemoglobin and contained only a trace of albumin

[7] Florence, Laura, *Am. J. Dis. Child.*, 1922, xxiii, 132.

with one or two exceptions. The liver cells usually contained more or less visible fat. The spleen was normal in size and flabby. The thoracic organs presented nothing characteristic.

Coming to the bacteriology of these cases we meet a definite condition in all animals. The spleen, liver, and kidneys contained large numbers of *Bacillus coli*. Each loopful of tissue fluid contained hundreds of bacteria. There was undoubtedly to a certain extent

TABLE I.

The Results of Feeding and Withholding Colostrum.

No. of calf.	Colostrum fed +; not fed −.	Source of dam.	No. of pregnancy.	Source of milk fed.	Date of birth.	Died, killed, or sold.	Remarks.
663	−	Eastern.	1	601	Oct. 4	Died, Oct. 6.	*B. coli* septicemia.
664	+	Native.	1	600, 601	" 10	Sold, Nov. 2.	
665	−	Western.	1	601	" 11	Died, Oct. 16.	*B. coli* septicemia.
669	+	Native.	1	601	" 18	Killed, Feb. 13.	Normal.
680	+	"	1	678	Nov. 1	Sold, Dec. 10.	
681	−	Eastern.	3 (?)	?	" 2	Killed, Nov. 5.	*B. coli* septicemia.
685	−	Native.	3	?	" 9	Died, " 11.	" " "
687	+	Western.	1	679	" 9	Sold, Dec. 10.	
694	+	Eastern.	1	679	" 25	Died, Jan. 2.	No cultures. Poison (?).
697	−	"	3	679	" 29	Sold, Feb. 2.	
698	+	Native.	4	678	Dec. 6	" " 2.	
699	−	"	3	678	" 6	Died, Dec. 8.	*B. coli* septicemia.
715	+	"	1	712	Jan. 5	" Feb. 19.	Cultures sterile. Poison (?).
717	−	Western.	1	678	" 11	" Jan. 22.	*B. coli* septicemia.
716	−	Native.	3	712	" 11	Killed, Mar. 16.	Normal. Cultures sterile.
718	+	Western.	1	678	" 12	Sold, Feb. 2.	
757	+	Native.	3	678	" 23	Died, " 17.	Cultures sterile. Poison (?).
759	−	"	2	678	" 25	Killed, " 21.	Miscellaneous bacteriemia.
893	+	"	1	712	Feb. 9	" Mar. 28.	Normal.
894	−	"	1	712	" 9	Died, " 13.	*B. coli* septicemia.
895	−	Eastern.	4	678	" 10	" " 13.	" " "
566	−	Native.	1	678	" 10	" " 12.	" " "

multiplication after death but, as will be seen farther on, these bacilli are present before natural death in abundance. Other organs were not cultured except in several cases certain joints which also yielded *Bacillus coli.*

The calf (No. 682) which died within 2 days and whose stomachs contained no food presented the same *Bacillus coli* septicemia.

No. 681 is of interest. On the 4th day of life it was too weak to get up and take its food. It would have presumably died during the night and it was therefore killed. The kidneys were firmer than normal and deeply congested. Cultures showed the presence of large numbers of *Bacillus coli* in the spleen, liver, and kidneys.

One calf (No. 717) died when 11 days old of a *Bacillus coli* septicemia. Cultures from one tarsal joint contained only *Bacillus coli.*

One calf (No. 759) which survived became lame. Several joints were involved. It was killed when 27 days old. There was a purulent fluid and a fibrin mold in one joint. The kidneys presented numerous whitish sclerotic foci. Cultures from the spleen remained sterile. Those from the kidneys and liver represented mixed infections, those from the joint exudate pure *Bacillus coli.* Of the two remaining calves, one was sold to the butcher in fine condition when 2 months and 3 days old. The other was killed when 2 months and 5 days old. The organs presented no abnormalities.

In Table I the cases are arranged according to the dates of birth. It shows that the calves deprived of colostrum and the controls were well balanced as to season.

DISCUSSION.

The data presented in the foregoing cases lead to certain definite inferences. The calf deprived of colostrum lacks something which permits intestinal bacteria to invade the body and multiply in the various organs. The rapidity and duration of this multiplication determine the fate of the calf. In most cases a rapidly fatal septicemia is the result. When the resistance is greater life may be prolonged or the animal survive indefinitely. The surviving animal may completely subdue the invading bacteria and develop into a normal calf or else localizations arise which involve, in the cases

here presented, the joints or the kidneys or both organs as in No. 759. The joint lesions are due to a fibrinous and purulent exudation. The bacteria present belong to *Bacillus coli*. The end-result of the localization in the kidneys is a fibrosis limited to the cortex with localized destruction of the secreting tissue. This occurs in foci of varying extent. The largest seen were about 1 cm. in diameter. This pathological condition, which was observed in 1917 associated with pneumonia due to *Bacillus actinoides*,[8] may now be considered as partly cleared up and it remains to study the early stages leading to fibrosis. The precise etiological factor may be *Bacillus coli* or some organism associated with it.

The bacteria isolated from the various cases and which were present in very large numbers in those that died and in fairly large numbers in those that were killed and cultured within 2 hours belong to that variety of *Bacillus coli* which is non-motile, indole-producing, and which fails to act on saccharose. This particular type of *Bacillus coli* has been under observation since 1917 and has been frequently isolated from the small intestines of calves dying in the first few days of life.

The experiments described are in a sense fundamental and many subsidiary questions are left unanswered. One of these is the effect of the ordinary milk of the different cows fed, whether protective and if so to what degree. In the table, the cow's milk fed is indicated by a number and it will be seen by consulting this that the source of the milk is not a determining factor in the experiment. Another question to be answered is whether it might not be possible by using extreme and unremitting care to raise most calves deprived of colostrum and how many of these would develop scours, joint lesions, omphalitis, or pneumonia in due time.

In general it may be concluded that the function of the colostrum is essentially protective against miscellaneous bacteria which are harmless later on when the protective functions of the calf have begun to operate and accumulate energy. There appears to be no function inherent in colostrum which controls development or growth or which is essential to the starting of the mechanism of digestion,

[8] Smith, T., *J. Exp. Med.*, 1921, xxxiii, 441.

since calves not having had colostrum appear to do as well as the others when the infection has been overcome.

The results obtained clear up much of the mystery surrounding the mortality and morbidity of very young calves. They explain why disease may appear when there is no evidence of introduction from without, and why the causes of disease of young calves up to 2 months old have been referred to the first days of life and to prenatal infection, especially where infectious abortion is prevalent. They moreover point out why so many different kinds of bacteria have been regarded as responsible for calf scours. Obviously the most invasive of the flora of any herd will dominate the bacteriology and different organisms will predominate in different territories unless the interchange of animals is very active, in which case the same flora will probably be found in many herds. The results also explain mixed infections, since the portal of entry from the intestinal tract is open.

These results furthermore show the inadequacy of all attempts at prevention based on keeping the invading bacteria away. These live in the normal cow, possibly some in the udder itself, and must be considered ubiquitous. They also demonstrate the futility of administering specific serum unless the dominating pathogenic organisms of the herd are known and used in its preparation.

It is not claimed as a result of these experiments that all colostrum-fed calves will resist the various forms of disease described, for we know that they prevail in spite of normal feeding at birth. There may be degrees of virulence in the intestinal, respiratory, and udder flora of cows against which normal feeding is quantitatively inadequate. It so happened that in the experiments reported no highly virulent types were present and therefore the dividing line between the calves protected by colostrum and those not so protected was unusually well defined.

CONCLUSIONS.

All of ten calves which were permitted to take colostrum after birth survived. Eight out of twelve calves which did not get colostrum died and one was killed moribund. One calf, killed on the 27th day, harbored miscellaneous bacteria in its organs. The kidneys

were sclerotic and one joint diseased. Of the remaining two calves, one had transitory joint troubles, the other rhinitis. One was sold and the other killed when 2 months old. In the latter the organs were normal and sterile.

Notes on the Individual Calves.

Calf 566.—Male, born Feb. 10, 1922, at 2.30 p.m., of a native cow in her second pregnancy. Calf removed from the dam immediately after birth and taken to the Department buildings at 4 p.m. There it was dried off by gentle friction with cloths. The umbilical cord was treated with bichloride of mercury and powdered boric acid. Fed 1½ lbs. milk (of Cow 678) from a bottle in evening.

Feb. 11. Calf fed three times today, drinking in all 4½ lbs. of milk. Temperature 39.0–39.5°C.

Feb. 12. Temperature 39.2°C. early. Calf drank first meal. Died about 11 a.m. Refrigerated at once until Feb. 13. The autopsy showed little of significance except the following.

The fourth stomach contained a milk curd the size of a small apple, some smaller clots, and milky fluid. Punctiform hemorrhages in leafy portion. Rectum deeply congested. Liver cells contain much fat. Extensive subendocardial extravasations of blood. Urine taken from bladder contains a trace of albumin. Both metatarsal joints contain some light yellowish fluid and soft mucoid flakes consisting largely of polynuclear leucocytes.

Cultures from spleen, liver, and kidneys are overgrown with a heavy glistening layer which in subsequent dilution cultures proves to be the saccharose-nonfermenting *B. coli*. One of several tubes inoculated with the exudate from the tarsal joint contains numerous colonies of the same organism.

Calf 663.—Born Oct. 4, 1921, at 11.50 a.m., of an eastern cow. First calf since introduction into herd. Calf not allowed to suckle the dam, and removed to Department unit at 1.35 p.m. Fed 2½ lbs. of milk from Cow 601 at 5.30 p.m. Abundant discharge of meconium during the night. Fed next morning. The discharges became softer and were yellowish in color.

Oct. 6. The calf was weak, unable to get up, and still scouring. Respirations shallow, about 60 per minute. Opaque, whitish, mucoid discharge from left nostril. Calf died at 11 a.m.

Autopsy.—Weight 67 lbs. Umbilical cord shrunken to a dried black tape-like body. Hemorrhagic discoloration of sheath and suspensory ligaments of umbilical artery stumps. Umbilical vein patent within abdomen to liver. Contents a normal blood clot. Beneath epicardium around base of heart, some hemorrhages. Hemorrhages within heart valves (auriculoventricular).

Digestive tract: Rumen normal. Fourth stomach contains white curds and some opalescent fluid. Leafy portion sprinkled with faded petechiæ. Pyloric portion and valve deeply congested. Uniform deep congestion of entire length

of small intestine. Bits of mucosa examined microscopically show complete injection of the network of capillaries in the villi. Large intestine congested but less so than the small. Liver shows considerable intracellular fat. The spleen is flabby, somewhat congested. Kidneys deeply congested. Urine taken from bladder is faintly clouded, yellow, slightly acid. Contains a trace of albumin and no sugar. Specific gravity 1.026.

Cultures from spleen and liver indicate large numbers of *B. coli* evidently pure. From the contents of a loop of the small intestine dilutions spread on agar show the same type of colonies. Those from a liver culture replated found to be colon bacilli non-motile, not fermenting saccharose.

Calf 664.—Born Oct. 10, 1921, at 5.20 p.m., first calf of a native cow, A 945. The dam had been vaccinated with a living culture of *B. abortus* before being bred. The parturition was normal and a guinea pig inoculated with material from a uterine swab was negative for *B. abortus*.

The calf remained with dam until 8.20 a.m. next day when it was removed to an isolation unit at the Department and fed milk from Cows 601 and 600. The temperature taken twice daily fluctuated between 38° and 39°C. The calf remained well and was sold Nov. 2.

Calf 665.—Born Oct. 11, 1921, at 2.50 p.m., of a midwestern cow introduced into the herd Sept. 27. Calf removed immediately after birth to a Department unit at 3 p.m. and fed milk from Cow 601.

Oct. 12. Calf drank 5 lbs. of milk in morning and 4 lbs. in evening. The temperature was 39° and 39.3°C.

Oct. 13. Calf drank 4 lbs. of milk morning and evening. Temperature 39° and 39.4°C.

Oct. 14. Calf drank 4 lbs. in morning. Feces soft, yellow, streaked with blood. Evening food withheld.

Oct. 15. Given 4 lbs. of milk. Temperature 39.8°C. Calf had a chill. Respirations 48; pulse 100. Feces soft with fetid odor. Discharge from both nostrils, mucous in character. Temperature, 5 p.m., 40.2°C. Calf drank 2 lbs. of milk. Temperature 39.6° at 9 p.m. Calf can stand up but unable to move legs freely.

Oct. 16. Temperature 38.6–39.1°C. during the day. Calf takes 1 lb. of milk, is very weak, unable to hold up head. Feces soft, streaked with blood. Legs cold. Dies at 11 p.m. and is placed in refrigerator.

The autopsy made early next morning. Weight of calf 88 lbs. The only noteworthy feature is an intense congestion of the medullary portion of kidneys. Urine from the bladder has a specific gravity of 1.011, yellow, cloudy, alkaline, albumin 0.2 per cent Esbach; sugar absent.

Cultures from spleen, liver, and kidneys show large numbers of colonies of non-motile bacilli like those from Calf 663. They ferment dextrose and lactose but not saccharose.

Calf 669.—Male, born Oct. 18, 1921, 1.20 p.m., first calf of a native cow. Remained with dam until 5 p.m. Then taken to an isolation unit at the Depart-

ment. The dam had been vaccinated same as dam of Calf 664. Calf was not fed in the evening. It refused milk next morning. In the evening it drank 2 lbs. The milk came from Cow 601.

The calf continued normal with temperature fluctuating between 37.5° and 38.5°C. until Nov. 5, when the daily taking was discontinued.

The calf was killed Feb. 14, 1922. It weighed 225 lbs. No lesions were found.

Calf 680.—Male, born during the night of Nov. 1 to 2, 1921, first-born of a native cow. The calf, which had suckled, was removed in the morning to an isolation unit at the Department and fed milk of Cow 678.

From Nov. 2 to 4, the stools were very liquid. Some diarrhea was again noticed on Nov. 21 and 22. The temperature fluctuated between 38° and 39°C. at this time. Otherwise the calf remained well and thrifty until sold Dec. 10.

Calf 681.—Male, born Nov. 2, 1921, 2 p.m., of a native cow. Probably the third or fourth calf. Calf kept from suckling the dam and brought over to a Department unit at 4.30 p.m. Fed 2 lbs. of milk.

Nov. 3. Fed 3 lbs. of milk in the morning. Large mass of feces passed with tendency to become liquid. Discharge from nose. Temperature 38.7–39.0°C.

Nov. 4. Takes 3 lbs. of milk. Discharge from nostrils more profuse. Calf grows weaker and unable to stand later in day. Temperature 39.2–39.7°C.

Nov. 5. Calf very weak, refuses food. Is unable to get up. In this case it was thought best to kill the calf to eliminate postmortem changes. Killed by stunning and cutting vessels of neck. Weight 73 lbs. The autopsy was negative except for the following.

The fourth stomach contained a mass of coagulated milk about 8 cm. in diameter. The mucosa of this organ and of the small and large intestines normal. The liver cells contain considerable fat. The kidneys are slightly enlarged, very firm to the touch, and deeply congested throughout. Urine from the bladder is yellowish, somewhat cloudy, slightly acid, specific gravity 1.030, albumin 0.15 per cent. Sections of the kidney show a complete injection of the entire capillary system. Those of spleen show numerous hemorrhages in the parenchyma.

Cultures were made from spleen, liver, and kidneys as follows: A bit of tissue about the size of a pea or small bean was transferred to an agar slant, rubbed over the surface, and deposited in the condensation water. A platinum loop dipped into this condensation water was rubbed over the slanted surface of a second tube. The numbers of colonies in the first tubes were countless. Those from the second tubes were 3 for the spleen, 15 for the liver, and 6 or 7 for the kidney. Dilutions from an intestinal loop planted in agar yielded the same kind of colonies; namely, the saccharose-non-fermenting *B. coli*.

Calf 682.—Born Nov. 2, 1921, before 6 a.m., of an eastern cow in her third pregnancy. This calf weak at birth remained so and was unable to take food. It grew weaker and was killed when about 33 hours old, Nov. 3, 2.30 p.m., to determine the presence or absence of *B. coli* septicemia.

The autopsy showed the absence of any food in the stomachs. These still contained the fetal fluids, viscid, bile-stained. The mucosa of nearly the entire

fourth stomach and pyloric valve was sprinkled over with minute, irregularly star-shaped hemorrhages, about 1 mm. apart. No surface hemorrhage. The small intestine nearly empty, normal. The lowest 24 inches of large intestine sprinkled with punctiform hemorrhages merging into a deep universal congestion in the rectum. The liver cells contained much fat. Other organs appear normal.

The cultures from spleen, liver, and kidney showed coalescing layers of growth in those in which bits of tissue had been placed. Those inoculated with a loop of condensation water from the preceding contained 6 or 7 colonies (spleen), 1 colony (kidney), and 6 or 7 colonies (liver). Dilutions of contents of an intestinal loop spread over agar showed the same type of colony. The bacilli were of the saccharose-non-fermenting type.

Calf 685.—Male, born Nov. 9, 1921, at 2 p.m., of a native cow in her third pregnancy. Calf removed from dam immediately after birth. At 3.10 p.m. placed with a cow which had calved 5 days ago. Calf began to suckle at 3.20 p.m. At 4 p.m. it was taken to a Department unit.

Nov. 10. Calf took 3 lbs. of milk early. Passed dark greenish masses of meconium. Feces tending to become liquid during the day. Temperature rose from 38.5°C. in morning to 39.6°C. in the late afternoon. Calf refused to take but a little milk late in the day, now very weak.

Nov. 11. Calf died at 5 a.m. From the autopsy record the following may be mentioned.

The umbilical vessels show only the usual amount of hemorrhagic discoloration. Rumen empty, normal. Fourth stomach normally distended with an opalescent, watery fluid and many small casein clumps. An area about 10 cm. in diameter of the leafy portion sprinkled with surface hemorrhages. Faded deeper hemorrhages in rest of leafy portion. In the small intestine there are well defined areas of deep congestion through the entire tube. There are linear congestions on crests of longitudinal folds of large intestine. Liver cells contain considerable fat. Spleen flabby. Some papillæ of kidneys congested. Heart and lungs negative.

Cultures from spleen, liver, and kidney show the same invasion of *B. coli* as in the preceding cases.

Calf 687.—Male, born Nov. 9, 1921, at 4.15 p.m. Remained with the dam until Nov. 10, 11.10 a.m., when it was removed to the Department animal house. The dam had been brought from Michigan in October. The calf refused to take milk (from Cow 679) the first evening, but drank next morning and thereafter. The temperature fluctuated between 38° and 39°C., rarely falling as low as 37.5°C. Nothing abnormal was observed. On Dec. 10 the calf was sold to the butcher.

Calf 694.—Born Nov. 25, 1921, at 1.30 p.m. Dam an eastern cow. Not known how many calves preceded this one. There was some difficulty in getting the calf to suckle the dam. At 4 p.m. it was induced to take colostrum from two quarters for about 10 minutes. At 4.30 p.m. it was transferred to the Department units. The subsequent feeding occasioned no special difficulties.

Up to Jan. 2, 1922, the temperature maximum and minimum were 39.8° and 37.5°C. Most of the morning and afternoon readings fluctuated between 38° and 39°C.

The calf was found dead unexpectedly at 7 a.m., Jan. 2. It had not been seen since 11 p.m., Jan. 1. It was at once placed in the refrigerator until Jan. 3. The autopsy presented the following points.

The mucosa of the digestive tract was abnormally pale. The fourth stomach contained a firm milk coagulum in which were enmeshed pieces of twine eaten by the calf from the straw bedding. There was more than the usual bile staining in the small and large intestines. The heart cavities contained soft dark clots and the endocardium was deeply suffused with blood coloring matter. The spleen was very flabby and of normal size. Some large bacilli (postmortem?) present. The liver was yellowish in color. There were a moderate amount of fat in the liver cells and some orange pigment. Bacteria not seen in films. The kidneys on section showed closely set, pale, linear streaks, passing vertically through cortex. In fresh scrapings the nuclei of the convoluted tubule epithelium contained each an irregular, refringent block or concretion. The thymus within the thorax was densely studded with hemorrhages up to 2 mm. in diameter. The cervical portion was free from them. Cultures were not made.

Calf 697.—Female, born Nov. 29, 1921, 2 p.m., of a native cow in her third pregnancy. The calf was unable to stand up for a time. At 2.45 p.m. it was transferred to the Department units and gently dried by rubbing with cloths. At 4 p.m. it was allowed to suckle another cow (No. 600) but fed poorly and obtained little.

Nov. 30. Temperature 38.5° and 39°C. Took 2½ lbs. of milk morning and evening. Voids large masses of meconium. The milk came from Cow 679. Dec. 1. Temperature 39° and 39.7°C. Drank 3 lbs. milk morning and evening. Mucopurulent discharge from nostrils. Dec. 2. Temperature 39.3° and 40.2°C. Food as yesterday. Dec. 3. Temperature 40°C. Discharge from nostrils abundant, thick. Feces normal. Dec. 4. Temperature 39.3° and 39°C. Food as before. The calf soon recovered from the rhinitis and gained weight normally. It was used in another experiment and sold to the butcher in fine condition Feb. 2, 1922. The inspector reported organs normal.

Calf 698.—Male, born Dec. 6, 1921, between 12 and 1, noon, fourth calf of a native cow. The calf was allowed to take the colostrum and then removed at 2.20 p.m. to the Department buildings.

The calf refused to drink next day until evening when some milk was slowly poured into its mouth. At 11 p.m. it drank about 12 ounces from a bottle. On the following day it began to take food in larger quantities. The temperature rose to 39.6°C. on Dec. 7. Thereafter it fluctuated between 38° and 39.5°C., falling to 37.4°C., Dec. 15. Temperature taking discontinued after Dec. 23. Calf sold to butcher Feb. 2, 1922. Inspector reported the organs normal.

Calf 699.—Male, born Dec. 6, 1921, at noon, of a native cow in her third pregnancy. Calf removed at once to Department buildings and dried off by rubbing with cloths. Fed about 2 lbs. of milk from Cow 678.

Dec. 7. Calf drank about 2½ lbs. of milk morning and evening. A large amount of feces was passed during the day, gradually assuming a liquid condition. Temperature 38.2° and 39.7°C. Dec. 8. Calf died at 8 a.m. At the autopsy the following appearances were noted.

The mucosa of pharynx and larynx cyanotic. A milk curd about 3 to 3½ inches in diameter in the fourth stomach and some milky fluid. Mucosa dark bluish red. The mucosa of small intestine deeply injected throughout. Microscopic examination shows complete injection of capillary system of villi. The large intestine similarly congested with intensification in the rectum. Bowels practically empty. Thoracic organs show nothing noteworthy. Liver cells contain considerable fat. The spleen is splashed with subcapsular hemorrhages. The kidneys are deeply congested throughout. Urine taken from the bladder is clear, yellow, acid. Specific gravity 1.025. A trace of albumin present.

Cultures from spleen, kidney, and liver show as heretofore the presence of large numbers of colon bacilli, not fermenting saccharose. The same type isolated from two segments of the small intestine. Other species not in evidence.

Calf 715.—Female, born Jan. 5, 1922, about 4 a.m., of a native cow in her first pregnancy. The calf was left with dam until 4 p.m. and then brought to the Department units.

The calf was fed milk from Cow 712 the next day and feeding continued three times daily. The temperature remained normal and the appetite good. From Jan. 18 on it was taking 12 lbs. of milk. Jan. 26. *B. bovisepticus* was introduced into one nostril and the reaction following was slight. It was found dead unexpectedly Feb. 19 at 5.30 a.m. when 45 days old. The watchman reported behavior of the calf similar to that of No. 757 before death. The calf was placed in the refrigerator until Feb. 20.

The only noteworthy changes observed at the autopsy were as follows: The fourth stomach contains milk clots mixed with twine picked out of bedding. The mucosa is partly digested. Extensive subendocardial hemorrhages in the left ventricle. All cavities contain soft dark clots. The spleen is very flabby and not enlarged. The liver cells contain large fat globules. The kidney cortex is regularly marked with grayish streaks. The intrathoracic portion of the thymus is permeated with hemorrhages 1 to 2 mm. in diameter.

Cultures of bits of tissue from liver, spleen, and kidneys in plain and blood agar slants and in fermentation tubes remain indefinitely sterile. Similarly, cultures from the heart's blood.

Calf 716.—Female, born Jan. 11, 1922, 1.15 p.m., of a native cow in her third pregnancy. Calf taken from cow and removed to the Department buildings, rubbed until nearly dry. Fed 1½ lbs. of milk from Cow 712 in evening from a nursing bottle.

Jan. 12 to 16. The calf takes milk regularly and is fed three times a day. The temperature is within the normal range. Jan. 17 to 18. Temperature 39.5°C. Evidence of joint involvement in left elbow and tarsal joints. Calf constipated. Jan. 19. Calf moves more easily about stall. Is taking 9 lbs. of milk daily in three doses. Feb. 1. Slight lameness in left fore leg and some weakness in hind limbs. Feb. 11. Swelling of left tarsal joint probably a distension of synovial sheaths. Feb. 28. Temperature has been normal to date. The joint troubles have practically disappeared. This calf was killed Mar. 16, when 2 months and 5 days old. There were no abnormal appearances of the organs and the cultures made as heretofore remained sterile.

Calf 717.—Male, born Jan. 11, 1922, at 4 p.m., of a midwestern cow. First calf since coming into this herd. The calf was removed at 5 p.m. to the Department buildings and dried off by gentle friction with cloths. Fed at 5.30 p.m. from a nursing bottle about 1 lb. of milk from Cow 678.

Jan. 12 to 19. Fed three times daily about 2 lbs. of milk each time. Jan. 20. Temperature up to 40.2°C. this morning. Calf is very weak but is taking its food as usual. Constipated. Temperature 41°C. at 8 p.m. Respirations short and irregular. Jan. 21. Calf took its food three times today. Is slightly better. Umbilicus slightly swollen and tender. Jan. 22. Calf too weak to get up and take its food. Dies at 10 a.m. Refrigerated at once until next day.

Jan. 23. Autopsy. Weight of calf 92 lbs. Umbilicus has a dry black tape still attached. Base slightly thickened and indurated. When tape removed, a circular area is exposed covered with a thin layer of pus and communicating with a closed diverticulum with purulent contents. No communication with umbilical vein, which is closed. The digestive tract normal with exception of some deeply congested patches of mucosa in ileum. Lungs slightly edematous and congested. Some small fibrin masses in pericardial sac and a few hemorrhages under epicardium. Liver, spleen, and kidneys softer than normal. Slight vascular injection of tissues within tarsal joints. Urine from the bladder contains only a trace of albumin. Specific gravity 1.012.

Cultures from spleen, liver, and kidneys show presence of large numbers of *B. coli.* Many colonies of the same bacilli in pure culture obtained from the fluid in one tarsal joint.

Sections of fixed and hardened tissue presented the following data. Spleen: Foci or colonies of *B. coli*-like rods scattered regularly through the section. Liver: One minute abscess in the section. Every liver cell contains one or more (fat) vacuoles. Each vacuole contains a central coccus-like body stained reddish (fat crystals). Kidney: Fibrin thrombi in small vessels of cortex. Occasional foci of polynuclear cells, interstitial hemorrhages, and *B. coli*-like rods filling capillaries.

Calf 718.—Male, born Jan. 12, 1922, at 7.45 a.m. Dam brought from Michigan Nov. 20, 1921. To make certain that the calf had received colostrum it was placed with the dam again at 11 a.m. and allowed to suckle the four quarters, in

all for 20 minutes. It was then brought to the Department buildings. The milk fed was from Cow 678. No difficulty was experienced in starting the artificial feeding. The maximum and minimum temperatures until Feb. 2 were 38.8° and 37.8°C. On this day it was sold to the butcher. Organs were reported normal by inspector.

Calf 757.—Male, born Jan. 23, 1922, at 9 a.m., of a native cow in her third pregnancy. The calf suckled the dam about 15 minutes before removal to the Department units. The cord was treated in the same way as that of Calf 893. The calf was fed three times daily with milk of Cow 678. The temperature and appetite continued normal. On Feb. 13, being in fine condition, it was used in another experiment, *B. bovisepticus* was introduced into a nostril. The reaction was slight.

On Feb. 17, when the calf was 22 days old and had been without abnormal symptoms thus far, it suddenly (1.55 p.m.) became very excited. It moved in a circle from left to right and lowed continually. The respirations were noisy, labored, the tongue protruded slightly, and there was more or less frothy mucus about the muzzle. The calf was forcibly restrained by being thrown and held. After a time the convulsive movements became less pronounced and the dyspnea more marked. It soon was unable to rise after being released and died 15 minutes after it had been discovered in this condition. This calf up to the time of the seizure had been in excellent condition and had even taken its milk early on the day of death as usual.

The autopsy showed nothing abnormal, except numerous small hemorrhages through the intrathoracic portion of the thymus and some minute hemorrhages under epicardium. The fourth stomach contained pieces of twine in the milk coagulum.

Cultures on plain and blood agar slants with bits of tissue from spleen, liver, and kidneys and with heart's blood, sealed, remained free from growth after 5 days incubation.

Calf 759.—Male, born of a native cow in her second pregnancy, Jan. 25, 1922, 3.20 p.m. Removed from the dam before suckling and taken to the Department buildings where it was dried off by gentle rubbing with cloths. The umbilical cord had been torn off close to the skin. The exposed area was washed with 0.1 per cent mercuric bichloride and dusted with powdered boric acid. The calf was very weak and scarcely able to stand up. Fed from bottle ¾ lb. of milk from Cow 678. Fed again at 10.35 p.m.

Jan. 26. Fed three times today. Dry meconium being passed. Calf stronger. Jan. 27. Temperature 39°C. Takes food regularly. Soft yellow feces passed in large amounts, becoming liquid. Jan. 28. Temperature 39.1–39.2°C. Feces liquid. Jan. 29. Temperature 39–38.4°C. Lameness appears in right carpal and left tarsal joints. Calf quite weak. Feb. 4. Temperature has ranged close to 39°C. Some swelling of right carpal joint and stiffness in hind limbs.

Feb. 21. Calf has been improving and taking about 12 lbs. of milk daily. Killed today by stunning and cutting vessels of neck. Weight 87 lbs.

The autopsy showed the following abnormalities. In the right cephalic lobe of lungs some collapsed areas, each 4 to 5 mm. in diameter. Both kidneys spotted with white patches varying from mere specks to areas of a square centimeter. These areas correspond to the bases of solid foci reaching to medulla. A vertical section through these foci is either linear, square, fan-shaped, or oval. The involved tissue is white, smooth, glistening on section and quite firm. The distribution is irregular, not associated with any structures in the cortex. The urine taken from bladder is clear amber-colored, alkaline, specific gravity 1.020. A trace of albumin is present. The liver shows a distinct fatty zone on the periphery of the lobules.

In the right radiocarpal articulation a small quantity of an opaque fluid containing soft mucoid flakes together with a firm whitish fibrinous mold of a portion of the joint cavity, loose, and easily removed with forceps. The exudation and edema extend from the joint up the external aspect of the leg (radius) between the muscles for 3 to 4 inches. The joint exudate consists of polynuclear leucocytes.

Cultures were made with bits of tissue from spleen, liver, kidney, and the diseased joint. The spleen culture remained sterile. In the liver and kidney tubes several species of bacteria appeared, indicating a miscellaneous infection of the body. The joint cultures contained *B. coli*.

Calf 893.—Male, born Feb. 9, 1922, about 6 a.m. First-born of a native cow. Calf at first removed from dam as not having suckled, but owing to uncertainty in this respect, it was allowed to suckle dam at 11 a.m. for 20 minutes and then taken to the Department buildings. The umbilical cord was snipped off, leaving about 1 inch. This was dipped in 0.1 per cent mercuric chloride and covered with powdered boric acid. First meal taken the same evening was milk from Cow 712. There was no difficulty in feeding. The temperature was taken twice daily until March when the calf was used in another experiment. During this time, the maximum and the minimum temperatures were 39.4° and 37.7°C. The calf was killed Mar. 28. The only abnormality found was pigmentation of the mucosa of cecum, along the course of the larger vessels.

Calf 894.—Male, born Feb. 9, 1922, 6.30 a.m., of a native cow in her first pregnancy. The calf was removed from the dam but was not taken to the Department buildings until about noon. It was probably exposed and chilled in this interval. It was then gently rubbed with cloths and the umbilical cord treated as in earlier cases. Fed at noon and night from a bottle with milk from Cow 712. Feb. 10. Fed three times today. Temperature 38.8° and 39.2°C. Large amounts of dark greenish feces passed today. Feb. 11 to 12. Temperature range 38.8–39.4°. Tendency towards diarrhea. Feb. 13. Calf is very weak. Takes milk twice, but unable towards night to stand. Feces fluid. Dies at 10.30 p.m. Placed in refrigerator until next morning. Weight 73 lbs.

Autopsy negative except for petechiæ in fourth stomach and a deep congestion of the mucosa of ileum. Tubes inoculated from spleen, liver, and kidneys show large numbers of colonies of *B. coli.*

Calf 895.—Male, born about 7 a.m., Feb. 10, 1922, of an eastern cow in her fourth pregnancy. Removed promptly from the dam and taken to the Department units at 10.20 a.m. where it was dried and umbilicus treated. The calf was fed at noon and evening with milk from Cow 678.

Feb. 11. Large amounts of relatively firm meconium discharged last night. Fed three times today. Temperature 38.8–39.7°C. Feb. 12. Fed as before. Calf growing weak and unable to stand later in day. Temperature 39.1–39.5°C. Feb. 13. Calf unable to stand up and drink. Nostrils filled with mucus. Temperature falling, 38.4–37.1°C. Died at 11 p.m. and refrigerated soon after.

Feb. 14. Autopsy presents the following points. The umbilical vein just beneath abdominal wall is distended with a normal clot about 2 cm. in diameter. No communication with exterior. Fourth stomach contains some curds and milky fluid. Many superficial hemorrhagic points in leafy portion. Rectum deeply congested. Some dried yellowish fecal matter in it, blood-stained. Both carpal joints contain several cubic centimeters of a turbid fluid with flakes made up of polynuclear leucocytes.

Interarticular surfaces of tibiotarsal joints deeply congested. Cultures from spleen, liver, and kidneys show coalescent layers of growth. Cultures from metatarsal joints, large numbers of isolated colonies. These and the growth from spleen were replated and found to be the same type of *B. coli.*

INFLUENCE OF GLANDS WITH INTERNAL SECRETION ON THE RESPIRATORY EXCHANGE.

IV. Effect of Suprarenal Insufficiency in Cats.*†

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Marine and Baumann in 1921 demonstrated a significant and sustained increase in heat production in the majority of rabbits following suprarenalectomy or freezing the suprarenals (1). The importance of this type of reaction in general physiology and special pathology makes it desirable to ascertain whether this relationship between the suprarenal gland and basal metabolism is peculiar to some feature of organization in this species. The literature contains no satisfactory evidence on this point. Golyakowski, working with dogs and utilizing an undescribed method of ligation, studied the respiratory exchange (2). He reported an increase in CO_2 and heat production in eight of twelve dogs. However, the importance to be attached to this observation is greatly diminished by the fact that the oxygen intake was not altered. On the other hand, Aub reported a decrease in heat production in three cats following suprarenalectomy but he did not investigate the effect of sublethal crippling of the suprarenals (3). No other references bearing in any way on this subject could be found.

The rabbit has relatively great normal temperature variations and it usually has accessory suprarenal masses, differing from the higher mammals in both points. The cat is a typical member of the latter group. The temperature is fairly constant and accessory

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suprarenals are rare. Therefore, total removal is followed by death usually within 2 to 5 days (4). Also in its diet and its fat metabolism the cat differs from the rabbit and resembles the organization of man. This point takes on special interest in view of the recent demonstration by Marine and Baumann that the thyroid function is essential for the increase in heat production during suprarenal insufficiency (5), taken in conjunction with the connection between the thyroid and the metabolism of fat pointed out by McCarrison (6). Therefore, in this report we wish to record the changes in heat production observed in cats following varying degrees of suprarenal injury produced by partial extirpation, vessel ligation, freezing, and various combinations of these procedures.

Methods.

Cats were kept in the laboratory under uniform conditions of régime and diet for at least 3 weeks before determination of the respiratory exchange and they were run in the respiration chambers several times for training during this period. The gaseous metabolism was measured with the Haldane apparatus as modified by Marine and Lenhart (7). The room temperatures were kept relatively constant. The maximum variation (with the exception of October 5) was 19–24°C. with the mean of 21°C. Determinations were always made at least 15 hours after the last feeding; and a sufficient number of them over a period of at least 2 weeks was obtained to determine the normal range for the individual cat under the given conditions as regards season, food, state of nutrition, etc. About 10 per cent of the cats were rejected for this work because the control rates were too irregular. Those used were very quiet after becoming accustomed to the procedure, and frequently slept throughout the entire period in the metabolism chambers which was always 2 hours. Any periods in which the animal was restless were discarded.

Three methods were used in our attempts to cripple the suprarenal function:[1] (*a*) partial excision; (*b*) ligation of blood vessels; and (*c*) freezing.

Partial Excision.—This was the method first attempted as it would appear *a priori* to be the most exact means of producing varying

[1] All operations were performed under ether anesthesia.

degrees of suprarenal insufficiency. However, exactly the opposite was found to be the case. The leeway between the production of any effect and of a fatal insufficiency is extremely slight and uncontrollable. The removal of less than three-fourths of the suprarenal tissue produces no evidence of abnormality in heat production, while the result of removing more than this bears no necessary relationship to the anatomical amount remaining. Quite uncontrollable conditions, chiefly the blood supply of the remaining fragment, determine the outcome. By this method we were not able to produce any clinical change except the typical rapidly fatal symptom-complex of suprarenal suppression. This procedure was therefore abandoned.

Ligation of Blood Vessels.—For many years the fact has been known that occlusion of the lumbosuprarenal veins would produce marked histological changes in the suprarenal gland, particularly in the cortex (8). Hartman and Blatz, in 1919, reported clinical symptoms following ligation of the lumbosuprarenal veins which were suggestive of a functional suprarenal insufficiency as their basis (9).

Ligations of the suprarenal blood vessels were carried out in the following combinations: (*a*) right and left lumbosuprarenal veins; (*b*) left lumbosuprarenal vein with excision of the right suprarenal; (*c*) right and left lumbosuprarenal vein with mass ligation of the collateral renal-suprarenal vessels; and (*d*) left lumbosuprarenal vein with mass ligation of the collateral left suprarenal vessels and excision of the right suprarenal.

In ligating the lumbosuprarenal vein the peritoneum over it was slit just proximally and distally to the suprarenal gland and a silk ligature carried around the vein on an aneurysm needle without manipulating the gland or interfering with any of its nerve supply except those filaments that run in the sheath of the suprarenal vein. After ligation of the vein, congestion and swelling of the gland were observed to different degrees, the variation of which was extreme and unpredictable. Ligation by the same method in a series of animals gave entirely different results, depending on the collateral circulation which developed.

Freezing.—This method was developed by Marine and Baumann in their experiments to cripple the suprarenal glands in rabbits. They

pointed out the difficulties encountered in freezing the right gland and the relative ease on the left side. In the main the same situation prevails in cats except that the right suprarenal is somewhat more accessible, being usually less closely adherent to the vena cava. In some cases both glands were frozen, though more frequently the left was frozen and the right excised. Mobilizing the left suprarenal gland sufficiently to freeze it is very satisfactorily accomplished. The fatty areolar tissue is dissected back with the probe point for a distance of several millimeters so that the gland can easily be elevated with most of the nerves and minute blood vessels attached. A spray of ethyl chloride reaches all parts of the surface of the cortex. The duration of the freezing varies from 15 to 45 seconds, depending upon the degree of injury desired. There was difficulty encountered in obtaining ethyl chloride tubes having a spray sufficiently fine. Bengué tubes were the best available in this country at the time, and only about one in four of these was satisfactory. During the freezing the gland is blanched, but on thawing the circulation is seen to resume at once. To freeze the right suprarenal the lumbar approach is obligatory and the peritoneum is not opened, while for the left one either the lumbar or midline incision is satisfactory, and the suprarenal is reached transperitoneally. In either case the proper exposure of the suprarenal gland, effected by a fairly deep self-retaining retractor after the field is walled off and other structures are protected by sponges, is an important factor in controlling the degree of injury.

Controls.—Two types of control experiments were performed: (*a*) freezing tissue around the mobilized suprarenals, and (*b*) excision of one suprarenal gland.

(*a*) Two normal animals were subjected to operation in which both suprarenals were prepared as for freezing; the tissues about them were frozen but the glands themselves were not. This procedure included all the elements of shock, general trauma, and nerve and vascular injury that obtained when the glands were frozen, except for the actual injury to the cells of the suprarenal cortex.

(*b*) Five animals had one suprarenal excised, nothing being done at the time to the other gland.

In experiments in which thyroid function may be intimately concerned, the use of iodine for skin sterilization is contraindicated. Chemical sterilization of the skin by an alcoholic picric acid solution was the method used in these experiments.

Presentation of Data.

35 experiments were conducted on 24 cats in which the respiratory exchange was followed before and after operation, as follows: freezing, 10; ligation, 17; partial removal, 6; operative trauma, 2. The types of reaction to subtotal suprarenal insufficiency as observed in these cats may be roughly classified in three groups (Table I):

TABLE I.

Suprarenal glands.	Group I. No rise in heat production.		Group II. Rise in heat production.		Group III. Fall in heat production.	
	No.	Per cent.	No.	Per cent.	No.	Per cent.
Frozen	1	10	6	60	3	30
Ligated	7	41	6	35	4	24
Partial removal and trauma (controls)	7	88	0	0	1	12
Total	15	43	12	34	8	23

Group I. Those that live indefinitely and show no increase in basal metabolism.

Group II. Those that show a significant increase in basal metabolism (over 10 per cent).

Group III. Those that show a decrease in metabolism to a rapidly fatal termination.

There are variations in the reaction not specified in the above groups which will be referred to in the Discussion. No experiment has been considered to fall in Group II unless there was an increase of at least 10 per cent above the highest preoperative figure. The following six protocols have been selected as illustrative of the types of reaction observed.

Group I; Protocol 1.—Cat 10; adult, female (Table II). Aug. 29, 1921. Began metabolic determinations. Oct. 17. Under ether distally and proximally

ligated right and left lumbosuprarenal veins. Oct. 18. Active, eating well. Oct. 22. Normal in appearance. Oct. 24. Wound healed, stitches removed. Feb. 1, 1922. In stock; apparently in normal health.

Group II; Protocol 2.—Cat 7; adult, female (Table III). Sept. 2, 1921. Began metabolic determinations. Sept. 29. Under ether removed right suprarenal and froze the left. Oct. 1. Good appetite, alert, pupils dilated. Oct. 4. Marked diarrhea; eating very heartily (double ration). Oct. 6. Eating 150 gm. of milk and 150 gm. of meat each day and still seems hungry. Head feels

TABLE II.

Cat 10.

Date.	Weight.	O_2	CO_2	R.Q.	Total No. of calories.	No. of calories per kilo per hr.	Remarks.
1921	*gm.*	*gm.*	*gm.*				
Aug. 29	3,261	4.230	4.280	74	13.91	2.13	Control.
Sept. 2	3,138	3.805	3.825	73	12.58	2.00	"
" 14	3,148	4.485	4.520	73	14.86	2.36	"
" 16	3,154	3.655	3.955	79	12.21	1.94	"
" 30	3,198	3.730	4.090	80	12.49	1.97	"
Oct. 6	3,112	4.660	4.920	77	15.50	2.49	" (slightly restless).
" 10	2,972	4.065	4.250	76	13.53	2.28	"
" 13	2,818	3.860	4.050	76	12.89	2.29	"
Oct. 18	2,923	4.250	4.680	80	14.30	2.45	Oct. 17. Ligation of right and left suprarenal veins.
" 20	3,119	4.720	5.140	79	15.86	2.54	
" 22	3,181	4.400	4.750	79	14.66	2.30	
" 24	3,130	4.380	4.380	73	14.40	2.30	
" 26	3,204	4.900	5.430	81	16.43	2.56	
" 28	3,208	4.780	5.480	83	16.26	2.48	
" 31	3,185	4.540	4.870	78	15.18	2.38	
Nov. 3	3,328	4.480	5.130	83	15.22	2.29	
" 8	3,227	3.990	4.360	79	13.45	2.08	
" 11	3,060	4.230	4.310	74	14.02	2.29	
" 16	3,169	3.910	4.160	77	13.10	2.07	

warm. Oct. 27. Fur has become very glossy and smooth. Dec. 30. Sacrificed in good health.

Protocol 3.—Cat 18; three-quarters grown, female (Table IV). Sept. 26, 1921. Began metabolic determinations. Oct. 20. Under ether proximally and distally ligated right and left suprarenal veins. Oct. 22. Feels warm, eating well, active. Oct. 25. Not eating, very quiet. Oct. 26. Very weak, coughing occasionally. Oct. 27. Appetite returning. Nov. 18. Losing weight and strength, eating almost nothing, KI 25 mg. by mouth. Nov. 19. Very weak and

listless, scarcely holding up head; given emulsion of alcoholic extract residue from ox suprarenal cortex. Nov. 20. Very active and alert this morning, remarkable appetite, eating 100 gm. of meat without intermission; KI by mouth. Nov. 22. KI by mouth. Nov. 24. KI by mouth, gaining in weight, appearance improving. Nov. 25. KI 10 mg. Nov. 30. KI by mouth. Dec. 30. Sacrificed.

Group III; Protocol 4.—Cat 19; adult, female (Table V). Sept. 26, 1921. Began metabolic determinations. Oct. 7. KI 25 mg. by mouth. Oct. 29. Under

TABLE III.

Cat 7.

Date.	Weight.	O_2	CO_2	R.Q.	Total No. of calories.	No. of calories per kilo per hr.	Remarks.
1921	*gm.*	*gm.*	*gm.*				
Sept. 2	3,119	4.240	4.520	77	14.24	2.28	Control.
" 14	2,872	4.625	4.870	76	15.50	2.70	" (restless).
" 16	2,879	4.085	4.495	80	13.73	2.38	"
Sept. 30	2,891	3.910	4.250	79	13.12	2.28	Sept. 29. Right suprarenalectomy; left suprarenal frozen.
Oct. 2	2,784	4.410	4.480	74	14.57	2.62	
" 4	2,880	5.740	5.950	75	19.14	3.32	
" 6	3,028	5.710	5.950	76	18.94	3.13	
" 8	3,290	5.770	6.160	78	19.20	2.92	
" 10	3,242	4.920	5.080	75	16.33	2.52	
" 12	3,267	4.980	5.110	75	16.44	2.52	
" 15	3,405	5.010	5.200	75	16.72	2.46	
" 18	3,478	5.300	5.710	78	17.80	2.56	
" 20	3,675	5.760	6.250	79	19.29	2.62	
" 22	3,560	5.230	5.500	76	17.51	2.46	
" 24	3,506	4.900	5.010	74	16.29	2.32	
" 28	3,620	4.850	5.160	77	16.24	2.24	
Nov. 1	3,542	4.790	4.920	75	15.83	2.23	
" 8	3,202	3.670	3.930	78	12.25	1.91	
" 15	3,174	4.880	5.130	76	16.33	2.57	
" 21	3,444	4.560	4.670	74	15.18	2.20	

ether doubly ligated right and left suprarenal veins. Oct. 31. Very quiet and dopey in appearance. Nov. 2. Very weak, scarcely able to stand, feels cold, pupils dilated. Died during the night.

Autopsy.—Thyroid lobes very small, translucent, amber-yellow. Heart dilated, gray specks in pericardium; left suprarenal medulla is hemorrhagic, cortex necrotic. Three small, congested veins from posterior surface extending into perirenal fat and one into the renal vein. Right suprarenal is swollen but is not hemorrhagic,

TABLE IV.

Cat 18.

Date.	Weight.	O_2	CO_2	R.Q.	Total No. of calories.	No. of calories per kilo per hr.	Remarks.
1921	*gm.*	*gm.*	*gm.*				
Sept. 26	2,245	3.205	3.400	77	10.71	2.39	Control.
Oct. 5	2,203	3.810	4.160	79	12.84	2.91	" (temperature 16°C.; shivering).
" 10	2,047	2.585	2.770	78	8.63	2.11	Control.
" 13	1,951	3.115	3.365	79	10.43	2.67	"
Oct. 21	1,945	4.220	4.430	76	14.10	3.63	Oct. 20. Ligated right and left suprarenal veins.
" 23	1,937	3.770	4.090	79	12.62	3.26	
" 25	1,910	4.160	4.400	77	13.86	3.63	
" 27	1,937	3.430	3.710	78	11.56	2.99	
" 31	1,984	3.220	3.540	80	10.81	2.72	
Nov. 2	2,014	3.480	3.800	79	11.73	2.91	
" 4	2,001	3.730	3.980	78	12.41	3.10	
" 7	2,053	3.500	3.870	80	11.82	2.88	
" 9	2,147	4.200	4.640	80	14.18	3.30	
" 11	2,132	3.780	4.120	79	12.71	2.98	
" 14	2,156	3.480	3.920	82	11.74	2.72	
" 16	2,024	2.760	2.920	77	9.20	2.27	
" 18	1,894	2.190	2.390	80	7.29	1.92	
" 21	1,935	2.710	2.920	78	9.10	2.35	
" 22	1,988	3.340	3.820	83	11.33	2.85	
" 23	2,061	3.560	4.090	84	12.08	2.93	
" 25	2,110	3.190	3.550	81	10.74	2.54	
" 26	2,096	2.790	3.260	85	9.49	2.26	
" 28	2,228	3.670	4.250	84	12.49	2.80	
" 30	2,246	3.490	3.940	82	11.80	2.63	
Dec. 2	2,182	3.210	3.660	83	10.86	2.49	
" 5	2,077	2.530	2.760	79	8.52	2.05	
" 7	2,052	2.540	2.710	78	8.45	2.06	
" 9	2,022	2.450	2.670	79	8.24	2.04	
" 12	2,048	3.120	2.990	70	10.19	2.49	
" 14	2,090	2.730	3.120	83	9.26	2.21	
" 16	2,130	3.010	3.320	80	10.14	2.38	
" 20	2,214	3.240	3.770	85	10.98	2.48	

medulla appears intact grossly and microscopically, cortex yellowish white in color, microscopically necrotic. Abundant subcutaneous and abdominal fat. Other organs normal.

Protocol 5.—Cat 8; adult, female (Table VI). Aug. 29, 1921. Began metabolic studies. Sept. 29. Under ether removed right suprarenal and froze left. Sept. 30. Ate well. Oct. 1. Quiet and drowsy. Oct. 4. Very quiet, appears

TABLE V.

Cat 19.

Date.	Weight.	O_2	CO_2	R.Q.	Total No. of calories.	No. of calories per kilo per hr.	Remarks.
1921	*gm.*	*gm.*	*gm.*				
Sept. 26	2,089	3.245	3.515	79	10.85	2.59	Control.
Oct. 3	2,158	2.800	3.200	83	9.49	2.20	"
" 6	2,125	2.830	3.260	84	9.58	2.25	"
" 11	2,148	2.560	2.800	80	8.55	1.99	"
" 14	2,264	2.730	3.270	87	9.35	2.07	"
" 19	2,289	3.240	3.550	80	10.85	2.37	"
" 21	2,303	3.450	3.710	78	11.58	2.51	"
" 25	2,152	2.600	2.760	77	8.69	2.02	"
Oct. 31	2,040	2.410	2.380	72	7.91	1.94	Oct. 29. Ligated right and left suprarenal veins.
Nov. 2	1,938	1.150	1.350	85	3.93	1.01	Nov. 3. Died.

TABLE VI.

Cat 8.

Date.	Weight.	O_2	CO_2	R.Q.	Total No. of calories.	No. of calories per kilo per hr.	Remarks.
1921	*gm.*	*gm.*	*gm.*				
Aug. 29	2,412	4.135	4.320	76	13.75	2.85	Control.
Sept. 2	2,394	4.210	4.285	74	13.93	2.91	"
" 14	2,599	4.180	4.540	79	14.01	2.70	"
" 16	2,581	4.440	4.700	77	14.80	2.87	"
Sept. 30	2,719	3.530	3.980	82	11.92	2.19	Sept. 29. Right suprarenalectomy; left suprarenal frozen.
Oct. 2	2,649	4.400	4.510	75	14.51	2.74	
" 3	2,594	4.450	4.720	77	14.87	2.87	
" 6	2,502	3.080	3.240	77	10.21	2.04	
" 8	2,440	2.050	2.310	82	6.92	1.42	

dopey, alopecia, refuses meat, took 130 cc. of milk. Oct. 6. Lactose added to the milk; eating very little now. Oct. 7. Extremely weak. Lactose and milk 20 cc. by pipette. Oct. 8, p.m. Died.

Autopsy.—Abdominal and subcutaneous fat well preserved. Heart, normal color; heart's blood not coagulated. Right suprarenal absent; left embedded in omental adhesions, shows marked areas of necrosis in the cortex, medulla normal histologically. Stomach empty. Numerous erosions and hemorrhagic areas in mucous membrane.

Control. Protocol 6.—Cat 25; adult, male (Table VII). Oct. 28, 1921. Began metabolic observations. Nov. 5. KI 25 mg. by mouth. Nov. 8. Under ether mobilized both suprarenals and froze the tissue around them including blood vessels and nerves. Nov. 9. Normal appearance, eating well. Nov. 13. Normal behavior, marked dermatitis over shaved areas. Nov. 22. Wounds completely healed. Cat appears normal in every way.

TABLE VII.

Cat 25.

Date.	Weight.	O_2	CO_2	R.Q.	Total No. of calories.	No. of calories per kilo per hr.	Remarks.
1921	*gm.*	*gm.*	*gm.*				
Oct. 28	2,589	3.790	3.780	73	12.43	2.40	Control.
" 31	2,683	4.710	4.750	73	15.62	2.91	"
Nov. 3	2,880	3.990	4.630	84	13.61	2.36	"
" 8	2,955	5.280	5.850	81	17.70	2.99	"
Nov. 9	2,785	4.540	4.640	74	15.09	2.71	Nov. 8. Control freezing operation.
" 11	2,618	3.780	3.960	76	12.60	2.41	
" 13	2,612	4.470	4.870	79	15.03	2.88	Marked dermatitis.
" 15	2,570	3.600	4.000	81	12.10	2.35	
" 17	2,522	3.400	3.790	81	11.46	2.27	
" 19	2,562	3.500	3.710	77	11.69	2.28	
" 21	2,574	3.780	4.100	79	12.65	2.46	
" 23	2,571	3.530	3.920	81	11.86	2.31	
" 26	2,619	3.800	4.260	82	12.82	2.45	
" 30	2,888	4.870	5.030	75	16.18	2.80	
Dec. 13	2,834	3.900	4.600	86	13.28	2.34	

Group I.—In this group the animals survive indefinitely and there is no increase in heat production of over 10 per cent. Eight, or 30 per cent of the twenty-seven experiments following freezing or ligation, presented this type of response to operation. These usually showed no noteworthy symptoms. Within 24 hours they had regained a normal appetite and behavior. In two cases there was a decrease in heat production lasting 2 weeks or more, not leading

to the death of the animal and with a subsequent return to the normal rate. Examination of the condition of the suprarenals either at autopsy or at a subsequent operation showed large areas of uninjured cortex, on both gross and microscopic examination. The medulla appeared quite normal. It is worthy of note that only one cat out of a total of ten, in which the suprarenals were frozen, fell in this group, and in this one the dissection of the gland for freezing was unsatisfactory on account of an excessive number of collateral blood vessels—a result of a previous ligation; while following vessel ligation, seven of seventeen showed no significant change in heat production. In the latter there was a well developed collateral circulation to the renal vein, the perirenal vein, and the diaphragm.

Protocol 1 illustrates this type of reaction. Eight determinations of the basal metabolism had been made over a period of 45 days, with a maximum variation of + 14 per cent and − 11 per cent from the average of 2.18 calories per kilo per hour. Both suprarenal veins were ligated proximally and distally to the suprarenal gland. Following this the gaseous metabolism was determined eleven times in 30 days—every other day for the first 2 weeks after operation. At no time was there a significant departure from the preoperative range, the maximum increase being 3 per cent above the highest normal figure.

Group II.—This group includes those cats whose heat production following suprarenal injury is increased at least 10 per cent above the highest preoperative figure. Twelve, or 44 per cent of the twenty-seven freezing or ligation experiments, showed this type of reaction. The increase varied from 18 to 44 per cent above the preoperative average and from 10 to 30 per cent above the highest preoperative figure. With one exception the rise in basal metabolism lasted from 9 to 38 days before again reaching the preoperative level. The increase in heat production was absolute as well as relative and in every case the cats survived the effect of suprarenal insufficiency indefinitely.

Coincident with the increased basal metabolism there were clinical manifestations of improved nutrition. Similar manifestations have been reported by Marine and Baumann in rabbits. The surface temperature seemed increased; the hair, which was shaggy in-

mediately after operation, became very sleek; and the appetite was often greatly increased. In spite of a food intake of double the normal amount the weight frequently was maintained at a level below its preoperative normal, though in some cases the cat gained tremendously in weight. Diarrhea was frequently observed, usually occurring at the height of the increase in heat production. Respiration was increased in rate and depth and at times there was a marked change in attitude and behavior, alertness and restlessness being observed.

Protocols 2 and 3 show typical examples of this type of reaction after freezing and ligation respectively. Cat 7 had the right suprarenal removed and the left frozen. The heat production was increased 36 per cent, reaching its maximum on the 5th day after operation and being maintained above the preoperative average for 20 days. The appetite was greatly increased and the cat gained 900 gm. in weight during this period of increased heat production, even gaining in weight when its basal metabolism was + 30 per cent. The total heat production also was increased by 30 per cent even before this gain in weight occurred. Diarrhea was noted on the 5th day after operation. The wounds healed promptly and the fur became very smooth.

Following this phase of increased heat production a slight depression averaging 10 per cent below the normal usually occurs, lasting from 1 to 4 weeks. That this may be partly due to an exhaustion of the thyroid gland is suggested by two facts. Potassium iodide given in two cases during this period of depression was followed by a prompt return to the normal level. And in several instances the thyroid gland was found 30 days after saturation with potassium iodide to be markedly hyperplastic in cats which had shown an increased basal metabolism after suprarenal crippling. At the time of being sacrificed the heat production had become subnormal. In control animals no such rapid exhaustion of the thyroid was observed.

Cat 18 showed an increase in heat production reaching the maximum of 44 per cent on the 1st and 5th days after ligation of both suprarenal veins. 2 days after operation it had regained its appetite and activity; however, by the 5th day after the operation it had become very inactive, was weak, and had lost its appetite. Its general

condition gradually improved; the basal metabolism remained elevated for 25 days, on the 20th day after the operation being + 40 per cent. At this time the animal lost its appetite and became very asthenic, rapidly losing weight; the heat production fell to 20 per cent below the preoperative figure. KI was given on November 18; on November 19 it ate less than 10 gm. of meat and did not hold its head up. Emulsion of suprarenal cortex extract was given by pipette. On November 20 the animal was very active, running and jumping, and when given food ate 100 gm. of meat without pause. The basal metabolism rose continuously for 3 days to a point above the preoperative range, it then became subnormal for a week, after which it resumed the original level. At autopsy the right suprarenal was found markedly atrophied; from the left there had developed a collateral circulation to the renal vein and the perirenal tissue. Colloid had disappeared almost completely from the thyroid vesicles and the epithelium was hypertrophic in spite of the fact that the cat had received potassium iodide 30 days previously in several doses.

Group III.—In this group the metabolism decreases slowly or rapidly till death. Seven, or 26 per cent of the twenty-seven animals, reacted in this way after freezing or ligation. The decrease in metabolism is on the average about 25 per cent. In the fulminating cases the picture is exactly like that after excision of both suprarenals (10), and the animal dies in 2 to 5 days. Two of this group lived 9 and 13 days respectively.

Histological examination of the suprarenal glands showed in each case marked injury to the suprarenal cortex. After freezing, the medulla had quite a normal appearance except for some congestion; its cells maintained their chromophilic properties. After ligation there was often very extensive hemorrhage into the medulla, though in some cases it remained entirely uninvolved. But the cortex always showed extensive necrosis in the animals of this group after either freezing or ligation. In some instances the entire cortex appeared to be necrotic, in others some areas of cortical cells staining fairly normally were found.

One cat in which fully seven-eighths of the suprarenal substance was excised reacted in this manner, showing marked reduction in heat production, and died $2\frac{1}{2}$ days after operation. No viable supra-

renal cells could be found, and the vein leading from this remnant was completely thrombosed.

Protocols 4 and 5 are illustrative of this type of reaction. Cat 19 showed a decrease of 54 per cent 4 days after ligation of both suprarenal veins, and was unable to walk. It died the following night. Autopsy revealed hemorrhage into the medulla of the left suprarenal and marked necrosis in the cortex. Three small congested veins extended to the perirenal fat and one to the renal vein. The right suprarenal showed no hemorrhage into the medulla, which seemed normal, while the cortex showed large areas of necrosis. Cat 8 lived 9 days after excision of the right suprarenal and freezing of the left. The day after operation the heat production was 22 per cent below normal, it then rose on the 3rd and 4th days after the operation to the upper part of its normal range, thereafter falling sharply to 50 per cent below normal on the 9th day, and death occurred that night. There was increasing weakness so that on the 8th day the cat could not stand and anorexia was marked, though for the first 5 days it ate over 100 gm. of milk and some meat each day. Autopsy showed well preserved fat stores, right suprarenal absent, left suprarenal cortex with large areas of necrosis, and medulla with normal staining properties.

The animals suffering from a fatal suprarenal insufficiency, though living over a week, give definite evidence of cardiac weakness. The heart rate may become irregular, the mucous membranes are somewhat cyanotic, and the animal goes into collapse on slight exertion, which speaks strongly for a marked diminution in its cardiac reserve. At autopsy the heart is somewhat dilated and the musculature, especially of the right ventricle, is flabby.

Controls.—In the two cats in which both glands were mobilized and the tissues about the glands were frozen, the heat production after operation showed no increase. Also the five animals in which one suprarenal was removed showed no increase of 10 per cent above the preoperative level. That these animals could, however, show the typical rise in basal metabolism was demonstrated in three of these five cats by either a previous or subsequent operation.

DISCUSSION.

The difference in reaction to suprarenal insufficiency in cats and rabbits, as reported in the literature, seems now to be cleared up. The previous failure to observe the characteristic increase in heat production in cats was due to the masking of this by the more profound effect of entire suppression of suprarenal function. In the six cases in which death resulted within a week from the suprarenal insufficiency, the heat production was invariably found to be continuously diminished, while in the two in which the suprarenal injury caused death, though the animal lived over a week, heat production in one case on the 4th day reached its highest preoperative level, and in the other case on the 5th day exceeded it, being followed in each case by a fall to death. Marine and Baumann observed in a small percentage of rabbits, also, this decrease in heat production to a rapidly fatal termination, and in them no accessory suprarenals could be found. It seems established then that when the suprarenal insufficiency approaches closely the suppression of suprarenal function heat production falls and death rapidly ensues. This is apparently due to the fact that the suprarenal glands, either by addition or subtraction, influence the reactive medium of the body in some manner essential to the continuance of vital metabolic processes.

Suprarenal injury less severe than the above causes in the majority of cats a profound effect upon the calorific mechanism, characterized by a significant increase in heat production. Of the twenty cats indefinitely surviving the suprarenal injury, twelve, or 60 per cent, showed this rise in basal metabolism, while of the control seven experiments involving all the elements of traumatization and manipulation of the suprarenals without serious damage to the glands, in none did the basal metabolism vary from its normal range by 10 per cent. In every case evidence of an increase in heat production was present in the 1st week, though the maximum frequently was not reached until later—24 days after operation in one instance. The rise lasted on the average 18 days before again reaching the preoperative base-line.

The secondary waves in the heat production curve mentioned by Golyakowski as occurring in dogs, and by Marine and Baumann in

rabbits, were very well marked in several instances. Cat 18 illustrates this phenomenon, one peak being in the 1st week after operation and another in the 3rd week.

After a period of suprarenal insufficiency associated with a significant rise in heat production there is usually a fall to 10 per cent or more below the base-line. That this is not altogether the effect of long training is shown by the fact that if it is followed sufficiently long the normal rate is resumed. This phenomenon resembles the period of hypothermia which is frequently seen in the convalescence from any prolonged fever. That thyroid exhaustion may be partly responsible for it is suggested by the effects of KI and by the rapid production of hyperplasia of the thyroid in these animals.

Of the three methods for producing suprarenal insufficiency in cats, partial excision was entirely unsatisfactory. Vein ligation gave good results in some cases but the collateral circulation was a dominant factor in the result which could not be predicted or controlled. In our hands freezing offered the most satisfactory means of controlling suprarenal injury.

In addition to the effect on heat production there are clinical symptoms, some of which can be readily correlated with it. Increased appetite, which may or may not be associated with a gain in weight, sleekness of fur, and rapidity of wound healing are nutritional manifestations which can be readily explained on the basis of thyroid function. There are other phenomena, however, which suggest vastly wider interrelationships between the suprarenal gland and the body tissues. In fatal suprarenal insufficiency gastric ulcer was frequently observed in this series in confirmation of the observations of other workers (11). Also symptoms of disturbed gastrointestinal function, most often manifest as diarrhea, were usually evident during the period of suprarenal insufficiency, most frequently observed at the time of maximal increase in basal metabolism. In one case diarrhea with numerous small blood clots was noted. Furthermore, the profound asthenia of the animals developing in the cases of fatal suprarenal insufficiency bespeaks a fundamental function of the suprarenal in relation to some part of the nerve-muscle motor mechanism. That this is not due merely to restriction of food intake was shown by the two cats which survived a fatal in-

sufficiency for over a week. In both cases operative recovery was prompt but a secondary profound weakness manifested itself about the 4th day which was well in advance of the stage of marked reduction in food intake. Cat 8, for example, was so weak, a week after operation that it could not walk on a flat surface, yet rough measurement showed the food intake up to this time to be 75 per cent of the caloric requirement of the animal. Rapid depletion of the glycogen stores have been demonstrated by previous observers (12). And other metabolic disturbances apart from the mobilization and use of fat were suggested by the rapidity of emaciation and the extreme degree to which it may be carried without exhaustion of the fat depots. Cat 12, for example, lost one-fifth of the body weight in 13 days, yet had abundant subcutaneous and mesenteric fat at autopsy. Extensive studies of the protein and especially of the water metabolism would be necessary to clear up this phenomenon.

A great mass of literature has sprung up about clinical suprarenal insufficiency, all of it vague, indefinite, and, up to the present, without any scientific support in fact (4), as there has been no method of differentiating the degrees of sublethal suprarenal insufficiency. Comparative measurements of heat production in the presence of an iodized thyroid may possibly offer a means of following to some degree the various phases of insufficiency of the suprarenal cortex. This work with cats, as well as that of Marine and Baumann with rabbits, shows that the partial suprarenal cortex insufficiencies produced experimentally are transitory and that compensation takes place rapidly or the animal dies. Therefore, one would not expect to demonstrate a suprarenal insufficiency in animals that have survived the injury for months.

Further evidence of the importance of the cortex as opposed to the medulla in influencing essential tissue functions is indirectly afforded by these experiments. In all cases in which death ensued from the suprarenal insufficiency, whether induced by freezing or ligation, marked damage to the cortex was evident, while the medulla usually retained its normal appearance grossly and microscopically. Even though secretion of epinephrine is possibly suppressed by freezing, vessel ligation leaves the nerve supply which controls this secretion, for the most part intact. There is a path for its discharge—

a definite collateral circulation between the suprarenal medulla and the perirenal tissue having been reported by Cow in 1914 (13) after ligation of the suprarenal vein proximally and distally to the gland. The collateral venous circulation of the suprarenal becomes markedly increased if the animal survives. These enlarged veins can readily be traced to the perirenal tissue, not infrequently to the diaphragm, and usually one or two directly to the renal vein. If it could be shown that under these conditions epinephrine was still discharged, it would necessarily follow that this agent played no essential part in the increased heat production.

It is impossible to study the syndrome of suprarenal insufficiency in cats without being impressed by the points of similarity between it and some of the outstanding features of Graves' disease. The most prominent single phenomenon in each is a significant increase in heat production. Each has a phase of asthenia and exhaustion, with evidence of severe cardiac damage. Diarrhea and nutritional and weight changes are common to each. Hyperplasia of the thyroid is produced in each and is characteristic of neither. Marine and Baumann have shown that the thyroid is indispensable for the increased heat production following suprarenal injury in rabbits but not for the asthenia and fatal termination. And, finally, that rather unusual form of Graves' disease running a rapidly fatal course in a very few weeks with severe asthenia, prostration, and coma bears a striking resemblance to the effect of fatal suprarenal insufficiency when death is postponed for several days.

CONCLUSIONS.

1. Severe and sufficient non-fatal injury to the suprarenal cortex by freezing or by ligation in cats causes a significant and prolonged increase in heat production.

2. Lethal injury to the suprarenals by freezing, ligation, or partial excision in cats causes a fall in heat production.

3. Insufficient injury to the suprarenals by freezing, ligation, or partial excision in cats produces no significant alteration in heat production.

4. Further evidence of a close thyroid-suprarenal cortex interrelationship is indicated by the rapidity of thyroid hyperplasia and by the effects of KI after suprarenal crippling.

I wish to express my appreciation for the continuous advice and interest of Dr. David Marine throughout this investigation.

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THE EXPERIMENTAL PRODUCTION OF PERIARTERITIS NODOSA IN THE RABBIT WITH A CONSIDERATION OF THE SPECIFIC CAUSAL EXCITANT.

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Plates 6 to 14.

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In the early reports (1) of periarteritis nodosa there occurs considerable discussion as to whether or not the lesions constitute a disease *per se* or represent a peculiar invasion of the vascular structures either by the "virus of syphilis" or by certain known microorganisms. The subsequent opportunities of study, however, have as a whole established it as a specific infectious disease of unknown etiology exhibiting a selective affinity for the vascular system, to which the essential lesions are confined. The disease is characterized especially by entrance of the injurious agent into the periarterial tissues, and the adventitia and media of the smaller arteries, leading to acute exudation and subsequent degenerative changes. The process may extend in the intima, causing rupture of the elastic lamina and the formation of multiple true aneurysms, extensive thrombosis or actual vascular rupture with hemorrhagic extravasation, or false aneurysmal formations. Moreover, signs of chronic inflammation associated with proliferative or reparative changes are present in certain of the vascular lesions.

The literature on periarteritis nodosa contains descriptions of the pathology and of the symptoms of the disease and little reference to the etiology regarding which the main reference is to syphilis. *Treponema pallidum* has been looked for diligently in tissues stained by the Levaditi method, but in no instance has it been detected. Other bacteria too have usually not been found. It is true that Babes and Mironescu (2) described diplococci and streptococci in the arterial lesions, but they report also an instance of syphilitic aneurysm of the aorta in which diplococci were detected. Although cited as a demonstration of the occurrence of streptococci in the lesions, these authors apparently do not regard the condition of periarteritis nodosa as present

in their specimens. Longcope (3) observed "a few groups of cocci in chains" in specially stained preparations of the kidney tissue distinct from the nodules; and absent from other involved organs.

The bacteriological investigations included blood cultures made during life and cultures at autopsy from the heart, pericardial cavity, gall bladder, peritoneal cavity, and the nodular lesions themselves. Beitzke's (4) *intra vitam* blood cultures were negative. Baehr's (5) and Manges and Baehr's (6) aerobic and anaerobic cultures made from blood during life and nodules excised at operation were also negative. Lamb (7) studied two cases and isolated various microorganisms from different organs and a streptococcus from a nodule. He inoculated, with negative results, with portions of the nodules and the streptococcus a number of guinea pigs, rabbits, and a monkey. Klotz (8) studied two cases and obtained *Staphylococcus aureus*, *B. proteus*, streptococci, and a diphtheroid bacillus from the heart's blood, bile, subcutaneous nodules, and the liver. He injected streptococci about the periarterial tissues or lymph spaces and induced certain lesions which he specifically states were not those of periarteritis nodosa.

The observations of von Haun (9) are more important. He injected intraperitoneally into two guinea pigs the blood of a proven case. The animals showed no evidences of the disease but died after 8 weeks. The heart's blood of these animals was injected into other guinea pigs and an organ emulsion, prepared from these animals, was injected into other guinea pigs. The second series of guinea pigs showed microscopic changes in the smaller arteries of organs and a disseminated inflammatory lesion of the adventitia and media of different arteries. Von Haun concludes that periarteritis nodosa is a specific infectious disease due to an unknown etiological factor present in the circulating blood and capable of inducing inflammatory changes in the walls of the blood vessels of guinea pigs.

Human Material.

The material employed in the present study was derived from a typical case of periarteritis nodosa admitted into the Charity Hospital of New Orleans on March 28, 1921. The patient was a colored male laborer, 32 years of age, a native of Louisiana. He had become ill 1 week before admission, suffering from general weakness, pain in the epigastric area and lower abdomen, and vomiting. The general physical examination with the exception of the abdominal tenderness was negative. The temperature chart showed an occasional rise above normal from 99–100°F. The pulse ranged from 80 on date of admission to 112 shortly before death. The respirations varied from 20 to 32. The Wassermann reaction was negative. The leucocytic counts ranged from 10,000 to 16,600 and the neutrophils from 71 to 80 per cent. Only one urinalysis is recorded, which was negative.

Radiological examination of the alimentary tract showed hyperperistalsis of the stomach, and pyloric spasm and hypermotility of the duodenum. Death occurred on April 29.

The autopsy was performed 72 hours after death. The body had been kept in the refrigerator at approximately 0°C. Only the anatomical structures showing pathological changes are described.

Peritoneal Cavity.—The coils of the small intestine are found adhered on the right side to a mass about the size of a cocoanut, consisting of a blood clot surrounding the right kidney. Another large clot is present beneath the omentum, and numerous more recent smaller clots between the coils of the intestines.

Stomach.—The gastroepiploic artery presents two large fusiform aneurysmal dilatations measuring respectively on cross-section 1.5 and 2 cm. in diameter. At about the middle of this vessel is a small branch which also shows a small nodule. In two of the nodules on cross-section there is a more or less pale red crescentic mass which on serial sectioning becomes smaller and finally disappears as the normal portion of the vessel is reached.

Kidneys.—When the previously described large blood mass is removed, the source of the hemorrhage can be traced to a ruptured aneurysmal sac within the outer portion of the cortex of the right kidney. The external surface of this kidney presents multiple bizarre shaped areas of mottled red and buff color. These areas are composed of arboriform injected vessels, some of which have ruptured, giving rise to petechial extravasations. Some areas contain pale centers and suggest infarcts. The sectioned organ (Fig. 1) shows extensive pathological changes. Numerous more or less rounded nodules from cream-white to garnet color and varying from 2 mm. to 1.5 cm. in size are found scattered throughout the kidney but are most prevalent in the cortical portion and the columns of Bertin. Upon serial section they terminate abruptly in the original vessel from which they arise and which in certain instances is so small as to be visible with difficulty or only with the hand lens. With the exception of the blood clot, the left kidney presents the same condition but to a less degree.

Heart.—The heart weighs 480 gm. Scattered irregularly along the course of the coronary arteries and their rami are found multiple

discrete nodosities varying in size from 2 to 6 mm. which present the appearance of partially filled pea-pods. They are for the most part of a yellow or tan color but a few are mottled with deep red and still others are entirely red. Upon section the nodules show irregular lamellations of dirty white and red color. Like those of the kidney they terminate abruptly and in some instances the vessel with which they are connected is found with difficulty.

Brain.—There are present a few small areas of sclerosis in the vessels of the circle of Willis. Neither these nor the other vessels of this organ, however, show the nodular changes described in the other organs.

Microscopic Examination.

Besides the structures presenting gross lesions, certain arteries in the liver and afferent arteries to the mesenteric lymph nodes present the microscopic changes of periarteritis nodosa.

The lesions may be classed into three groups: (1) the inflammatory reaction, or injury present in the vessel walls; (2) the resultant vascular lesions arising from those of the first group, namely aneurysms both true and false, thrombosis, and actual vascular rupture with hemorrhage; and (3) retrograde lesions occasioned in the parenchymatous structures or other tissues through the impairment of blood supply of these parts chiefly because of the lesions of the second group.

In the first group the changes are present especially in the adventitia and media. Varying appearances are observed. In some vessels they are purely periarterial and adventitial lesions showing polymorphonuclear neutrophils and fibrin. In other arteries this process is associated with a zone of necrosis in the media, forming at times a complete ring extending well down to the intima with fragmentation of the elastic membrane. In still other vessels the acute cellular and fibrinous exudate extends through the media down to the subendothelial layer (Figs. 2 and 3). At times the cells in the outer vessel area consist of lymphoid or plasma cells and eosinophils. In still other vessels cellular elements are absent and only necrosis of the media is found. Occasionally in these various lesions detachment of the endothelial cells or proliferation of these cells or of the subendothelial structures is noted. The proliferative processes are, however,

especially present in the dilated arteries in which thrombosis and aneurysm formation have occurred. The lesions of the second group resulting from those of the first group consist of aneurysms both true and false, and thrombosis and hemorrhagic extravasations. In them the inflammatory cellular elements are usually absent but when present they consist especially of lymphoid and plasma cells. The third group is formed by the lesions of the tissues resulting from the previous pathological processes. They are all of retrograde character and dependent upon the deprivation of the blood supply or the result of pressure from the hemorrhagic outbursts. Different kinds of degeneration, including necrosis with karyorrhexis or karyolysis, are found in the structures in which interference with the blood supply has been produced; and numerous infarcts of different sizes occur.

The gross and microscopic lesions demonstrate the existence of the peculiar pathology of the arteries characteristic of periarteritis nodosa. From the gross standpoint, the greatest intensity of the process is in the vessels of the kidneys. The branches of the renal arteries present extensive aneurysmal formations and multiple thrombosis (Fig. 1). The rupture of one of the renal aneurysms through the cortical substance and capsule permitted the extensive hemorrhage, the immediate cause of the death of the individual. The proportionately large size of the aneurysmal dilatation of even the very small arteries, for example the vasa recta, is very striking; from a microscopic artery an aneurysm 1.5 cm. in diameter may ensue. Very large nodules are also present in the gastroepiploic artery. These lesions are more fusiform in shape than the nodules in the other situations. The lesions of the coronary arteries and their branches are multiple but are more uniform and smaller in size. The liver while presenting no gross pathological change shows lesions of the first group present in a few of the interlobular arteries. Microscopic changes are also present in certain of the arteries supplying the mesenteric lymph nodes.

Transmission Experiments.

Experiment 1.—After searing the organs the gross lesions of the kidneys consisting of several nodules of various types and sizes were dissected out with sterile instruments and fragments cultured in plain, glycerol potato blood, dextrose agar, and Löffler's blood serum. No growth was detected after 1 week. Portions of the nod-

ules were ground in a sterile mortar in sterile normal saline solution and after the coarser particles sedimented out, 2 cc. of the finer emulsion were injected into the ear vein of two full grown rabbits, A and B. Rabbit A died 2 months later.

Autopsy of Rabbit A showed gross lesions in the liver only. They consisted of a hemorrhagic nodule on the under surface, measuring 2 mm. in diameter, and of several diffuse hemorrhagic extravasations beneath Glisson's capsule and extending into the organ. Two small infarct-like areas were noted.

Under the microscope the lungs show thinning and rupture in places of the alveolar walls and a marked thickening of the musculature of certain arteries with here and there a perivascular invasion of cells of the lymphoid and eosinophilic types which affect only one portion of the vessel's circumference and extend slightly into the adventitia.

The liver shows marked accumulations of lymphoid, polymorphonuclear, and eosinophilic cells in the portal spaces, chiefly about the interlobular artery penetrating to the media, and within the connective tissue. Inside the lobules are occasional smaller clusters of polymorphonuclear neutrophil and lymphoid cells. In certain of the affected arteries the walls show degeneration of the media and dilatation and thrombosis formation (Fig. 7). The cells of the liver itself show parenchymatous and fatty degeneration.

In the heart an occasional artery is to be seen with extensive necrosis of the outer portion of the media in which are a few polymorphonuclear neutrophilic and lymphoid cells. Fatty degeneration of the myocardium also occurs.

Aside from marked cloudy swelling the kidneys show no changes.

Rabbit B was killed 18 days later after drawing blood from the heart for inoculation and cultural purposes. The aerobic, anaerobic, and Smith tissue cultures remained sterile. Only the liver shows a few small hemorrhagic extravasations to the naked eye.

Under the microscope, on the other hand, the arteries of the lung show marked hypertrophy (Fig. 8) and, in some, proliferation of the subendothelial layers producing nodules which protrude into the lumen. The adventitia about certain arteries contains cells, chiefly eosinophilic (Fig. 8) and fewer lymphoid and plasma cells.

The portal tracts show accumulations of lymphoid cells and proliferation of connective tissue cells, especially marked about the arteries although occurring diffusely also (Fig. 9). The media of some vessels is degenerated and the vessels are ruptured, leading to hemorrhage into the tissues (Fig. 13).

Experiment 2.—Portions of the kidneys, liver, and heart of Rabbit B were removed, macerated with sterile normal salt solution, and 2 cc. of the supernatant fluid were injected into the ear vein of two full grown rabbits, C and D.

Rabbit C died 1 month after the inoculation. At autopsy only an occasional hemorrhage is seen in the liver.

Under the microscope the lung arteries show dense nodules of polymorphonuclear neutrophil cells situated especially in the adventitia, some of which extend into the media (Fig. 6). In certain vessels destruction of the outer coat and media and cellular infiltration extending to the intima are present.

Rabbit D died 6½ months after the inoculation. At autopsy no gross lesions are found.

Microscopic examination of the lungs shows some arteries with greatly hypertrophied walls about which are a few lymphoid cells.

In the liver the interlobular arteries show necrosis of the media, dilatation, and cellular invasion of lymphoid cells. The portal veins in some areas are dilatated and surrounded with hemorrhages.

The kidneys show congestion of vessels and exudation of serum between Bowman's capsule and the glomerular tufts.

Experiment 3.—Rabbit E was injected into the ear vein with 2 cc. of a filtrate prepared from the organs of Rabbit B of Experiment 1 killed 2 months and 18 days after inoculation with the human nodules. The filtrate was prepared from the liver, spleen, and kidney, the suspension being passed through a Berkefeld filter N. This rabbit died 3 months later and showed the most typical lesions of all the animals studied.

The lungs present small hemorrhages, on the pleural surface especially. In the right lung are two round nodular masses of a deep red color (Figs. 4 and 5). One of these nodules, measuring 4 mm. in diameter, is protruded from the anterior surface, while the other, measuring 3 mm., is located on the posterior surface. They show no intermediate connection. Near the hilum of the left lung are two small gray glistening nodules measuring approximately 1 mm.

The vessels of the heart were followed throughout their course, but no lesions were visible to the naked eye.

The surface of the liver beneath the capsule shows several small irregular hemorrhages. On section the organ shows small dilated vessels containing thrombi here and there.

The pulmonary arteries show under the microscope striking lesions. In some the adventitia contains many polymorphonuclear neutrophils and in others the infiltrating cells extend into the intima, and necrosis of the media (Figs. 10 and 11) is present. In many other arteries there occur also nodular and circumferential periadventitial accumulations of neutrophils, eosinophils, and lymphoid and plasma cells which often extend as far as the intima (Figs. 14 to 17). Rupture of the artery in the human case leading to false aneurysmal formation (changes of the second group) is represented by these lesions which are evident to the naked eye (Figs. 4 and 5). Pneumonic inflammation of the organ is absent.

In the liver the interlobular branches of the hepatic artery exhibit varying lesions. As compared with the human case we can distinguish of the first group polymorphonuclear neutrophilic and lymphoid cells and degeneration and necrosis of the media. Of the lesions of the second group the organ shows necrosis with dilatation of the arterial wall with aneurysm formation and rupture and hemorrhage (Fig. 12). Of the lesions of the third group are noted degeneration and necrosis of areas adjacent to the aneurysm and hemorrhage.

The arteries of heart and of the kidneys show occasional periarterial adventitial nodules.

Experiment 4.—Rabbit F inoculated intravenously with 1 cc. of defibrinated blood from Rabbit B of Experiment 1 has survived thus far for 6 months and is still under observation.

A consideration of the experiments, excluding the incomplete one, No. 4, shows that gross lesions consisting of aneurysms and hemorrhages occurred in the liver and lungs of many of the rabbits. Those most typical (Figs. 4 and 5) appeared in the lung of a rabbit injected with a filtrate obtained from an animal previously injected with the emulsion of the human nodules. In all the rabbits microscopic changes of some degree of intensity resembling those occurring in periarteritis nodosa of man existed. They consisted of infiltrations of the adventitia and media and even of the intima with cells of different types—neutrophils, eosinophils, and lymphoid and plasma cells. Coincidentally, degeneration and necrosis of the media, dilatation of the vascular lumen, and thromboses occurred. The intima showed proliferative changes. The veins were only exceptionally affected.

DISCUSSION.

Von Haun (9) produced injury to the arterial walls in the guinea pig by injecting blood from a case of periarteritis nodosa during life, thus demonstrating the presence of an active agent even though culture studies of the blood yielded negative results. Von Haun did not pass the blood through a filter, but he observed that the effects in animals of the second series were greater than in the first, and the inoculations proved fatal in them in 2 to 3 months. Our most pronounced results also were obtained with a second series of rabbits. The filtrate employed for inoculation yielded no microorganism. Hence the active agent may be regarded as a filter passer—through Berkefeld filter N.

The disease periarteritis nodosa, because of its rarity and infrequent recognition during life, has afforded a small field for experimental study. Among 54 reported cases, less than one-fourth record evidences of search for an etiological agent, and among these in only four instances, including the present case, has transmission of the disease to animals been attempted. However, the hardened tissues have afforded opportunity for the application of special stains for bacteria and *Treponema pallidum*; but all cases so studied have given

negative results. Although *Treponema pallidum* has been excluded the majority of observers have concluded that the affection is an infectious disease. Indeed, it seems unlikely that syphilis, which occasions such frequent vascular injury, should so rarely present the lesions characteristic of periarteritis nodosa. We believe that the experiments reported in this paper constitute additional evidence for eliminating *Treponema pallidum* as the inciting cause of periarteritis nodosa.

The pathological character of the gross and microscopic lesions suggests a specific inciting agent. It is probable that the microorganism, aside from its selective affinity for the vessel walls, produces an injury of a nature such as to induce the formation of aneurysms and thrombi. Other vascular lesions occurring in man and produced experimentally in animals by various known microorganisms do not present these characters; of them many are exudative, degenerative, and proliferative in nature, but the peculiar pathology of periarteritis nodosa, so clear-cut and readily discerned, is wanting. Thus multiple mycotic aneurysms (10), Charcot's multiple miliary aneurysms of the cerebral arteries, and various infectious lesions of the arteries (11) have no real analogy to periarteritis nodosa; while the arterial lesions produced in animals with the streptococcus and other microorganisms by Klotz (8, 12), Boinet and Romary (13), and others are distinct from those of periarteritis nodosa. Duval (14) described certain vascular changes in the arteries of rabbits due to *Bacillus mallei*, but neither thrombosis nor aneurysm occurred. In experimental typhus fever in the guinea pig Olitsky (15) described a "periarteriolitis nodosa" previously observed by Nicol which is of interest here in that hemorrhagic extravasations arise and the etiological agent is said to be filterable.

Various opinions have been held as to the manner of invasion of the microorganism in periarteritis nodosa. As our inoculations were made intravenously, the infectious agent was placed directly into the blood stream. Hence it appears probable that it is introduced in the outer vascular coats *via* the vasa vasorum or the perivascular lymph spaces. It is undecided whether the initial injury arises in the media or the adventitia. That the blood is capable of conveying the agent is borne out also by the experiments of von Haun (9) who

employed the blood derived from the vein of a living case for his animal inoculations.

In the reports of Adler (16), Hill (17), and Pearce (18) upon studies of the arterial system of normal rabbits no lesions suggestive of periarteritis nodosa are recorded. Lüpke (19) and Jaeger (20), however, describe a spontaneous disease in stags apparently identical with periarteritis nodosa of man. The possibility of the lower animal being the natural host for this virus is therefore to be considered.

CONCLUSIONS.

1. Periarteritis nodosa is a specific infectious disease which is transmissible to the rabbit.

2. The lesions induced in rabbits are identical with those occurring in man and consist of exudative and degenerative processes within the walls of the smaller arteries resulting in aneurysmal formations and thromboses.

3. The microorganism inducing the disease is capable of going through a Berkefeld N filter and is therefore to be classed with the group of so called filter passers.

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EXPLANATION OF PLATES.

PLATE 6.

FIG. 1. Photograph of the kidney showing multiple aneurysmal vessels of varying sizes with thrombi. These represent most of the lesions described as nodules. Where the section has not traversed the aneurysm the nodular aspect can be appreciated.

PLATE 7.

FIG. 2. Photomicrograph of an area in the kidney demonstrating lesions in the two arteries, one cut obliquely and the other transversely. The obliquely sectioned artery shows an inflammatory nodule involving the upper end of the artery and diffusing into the periadventitial tissues; extensive necrosis of a branch and also of the lower end of the artery proper is present. In the lower edge of the picture the artery cut transversely shows extensive necrosis down to the intima with no inflammatory reaction. Between these arteries is seen another artery not involved.

FIG. 3. Photomicrograph showing a higher magnification of the inflammatory nodule present at the upper end of the obliquely cut artery in Fig. 2. The involvement of the intima is shown.

PLATE 8.

FIGS. 4 and 5. Photographs of the nodules present on opposite surfaces of the lung of Rabbit E, inoculated with a filtrate derived from the organs of Rabbit B.

PLATE 9.

FIG. 6. Artery present in the lung of Rabbit C, second generation, inoculated with an organ emulsion of a first generation rabbit. This vessel demonstrates invasion of the periadventitial tissues and the adventitia and outer media with cells composed especially of the polymorphonuclear neutrophilic variety.

FIG. 7. Necrosis of arterial wall, with dilatation and thrombus formation present in the liver of Rabbit A. This rabbit was inoculated with the nodule emulsion obtained from the human case.

PLATE 10.

FIG. 8. The marked hypertrophy of the arterial walls described for several of the rabbits is herein demonstrated. This vessel also shows the presence in the adventitia of eosinophils and a few lymphoid and plasma cells. The section is from the lung of Rabbit B which was inoculated with the human nodular emulsion.

FIG. 9. Periarterial accumulation of lymphoid cells and proliferation of connective tissue cells present in the liver of the first generation Rabbit B. The artery is greatly injured and compressed.

PLATE 11.

FIG. 10. Section of a longitudinally cut artery in the lung of Rabbit E, inoculated with a filtrate obtained from a first generation rabbit. There is present on the upper surface a periarterial nodule formation.

FIG. 11. High magnification of the nodule present in Fig. 10, showing the cellular infiltration with destruction of the media and elastic membrane. The process extends to the intima and consists especially of cells of the polymorphonuclear neutrophilic variety.

PLATE 12.

FIG. 12. Section of the liver of Rabbit E, inoculated with a filtrate of the organs of a first generation rabbit, showing arterial necrosis and aneurysm formation with rupture and hemorrhagic extravasation into the liver tissue and beneath Glisson's capsule.

FIG. 13. Section of the liver of first generation Rabbit B, showing hemorrhagic extravasation into the surrounding tissue.

PLATE 13.

FIGS. 14 and 15. Low and high magnifications of an artery in the lung of Rabbit E. This animal had been inoculated with the filtrate of the organs of a first generation rabbit. The distinct periarterial nodular formation is prominent. The nodules are composed especially of lymphoid and plasma cells, with a few eosinophils and polymorphonuclear neutrophils. The involvement of the media by this process can be seen in Fig. 15.

PLATE 14.

FIGS. 16 and 17. Low and high magnifications of another artery in the lung of Rabbit E. The pathological processes are similar to those shown in Figs. 14 and 15, excepting that the distribution is more circumferential.

FIG. 1.

(Harris and Friedrichs: Periarteritis nodosa.)

FIG. 2.

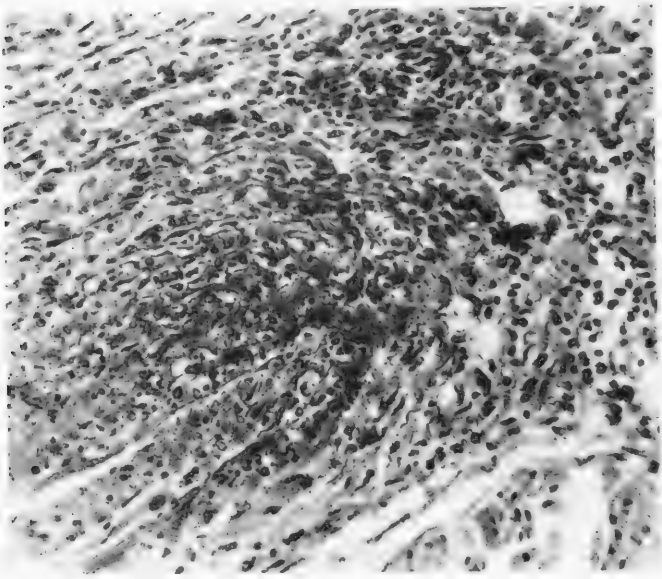

FIG. 3.

(Harris and Friedrichs: Periarteritis nodosa.)

FIG. 4.

FIG. 5.

(Harris and Friedrichs: Periarteritis nodosa.)

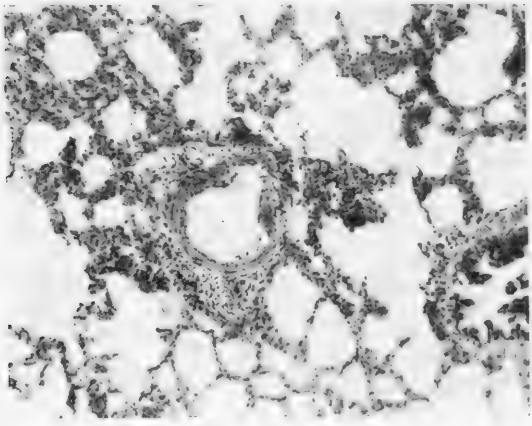

FIG. 6.

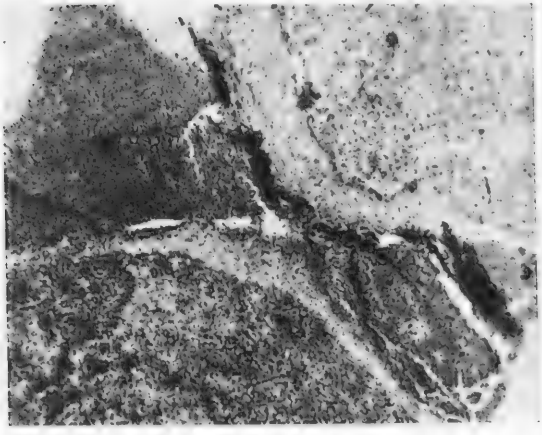

FIG. 7.

(Harris and Friedrichs: Periarteritis nodosa.)

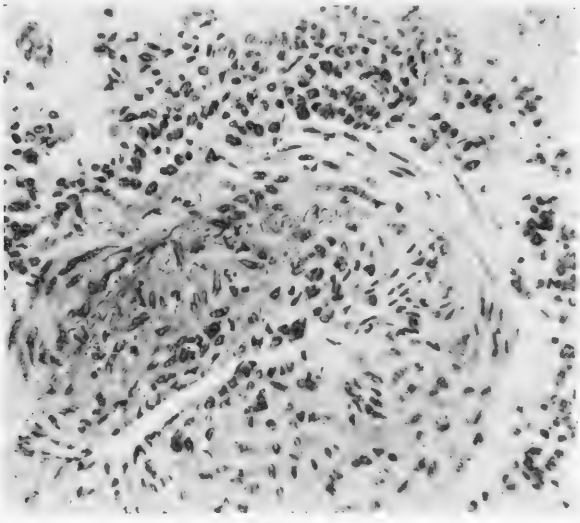

FIG. 8.

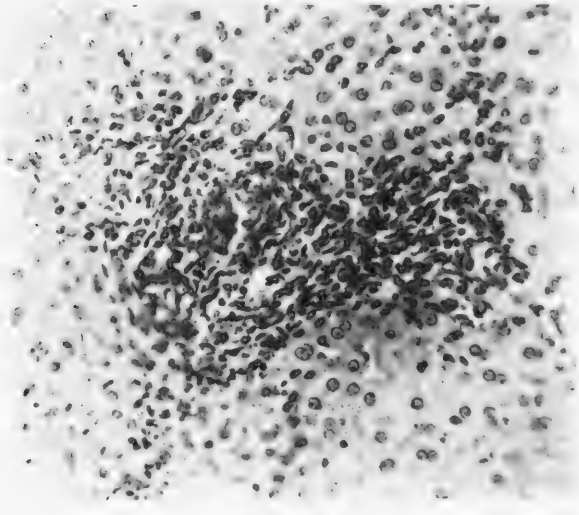

FIG. 9.

(Harris and Friedrichs: Periarteritis nodosa.)

FIG. 10.

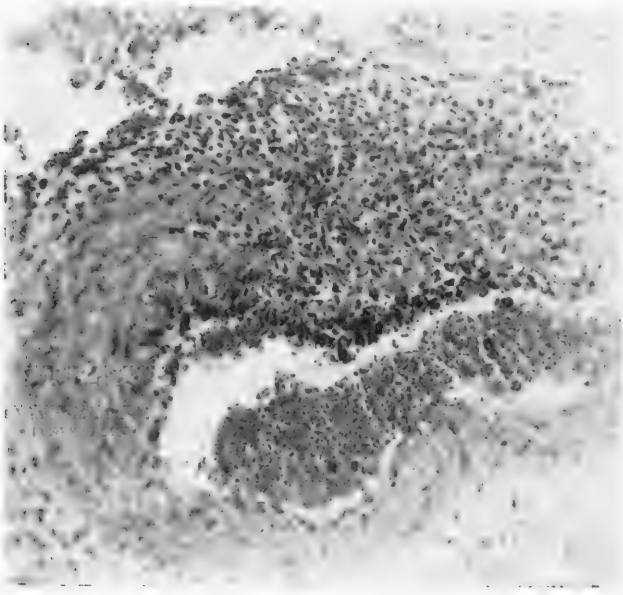

FIG. 11.

(Harris and Friedrichs: Periarteritis nodosa.)

FIG. 12.

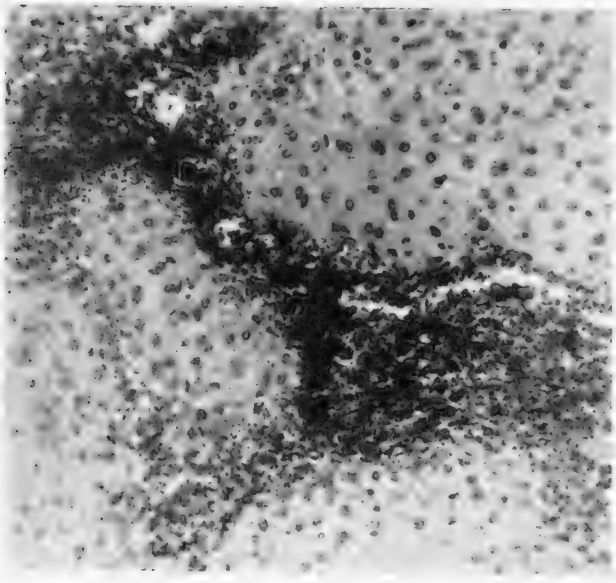

FIG. 13.

(Harris and Friedrichs: Periarteritis nodosa.)

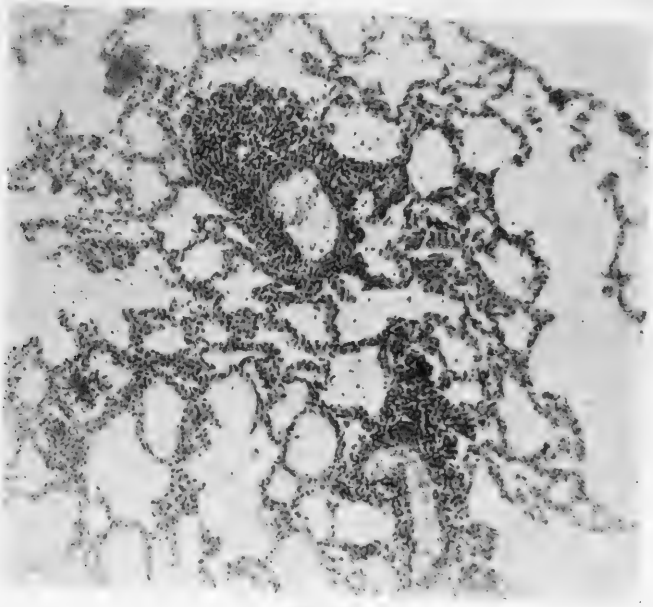

FIG. 14.

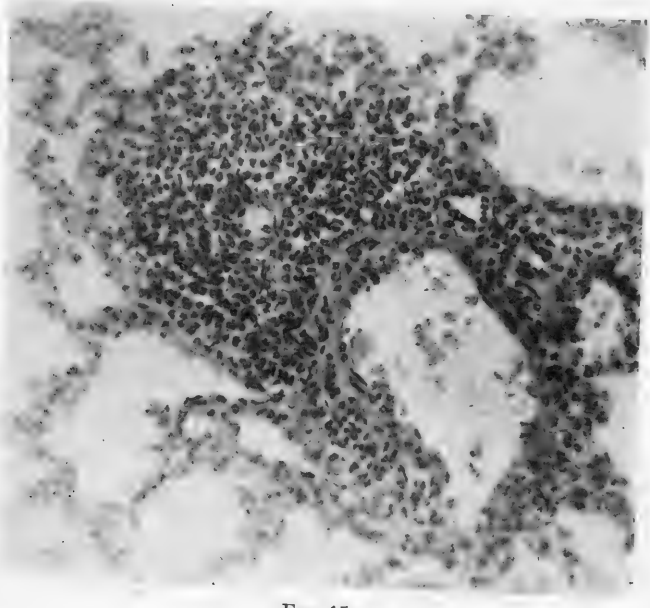

FIG. 15.

(Harris and Friedrichs: Periarteritis nodosa.)

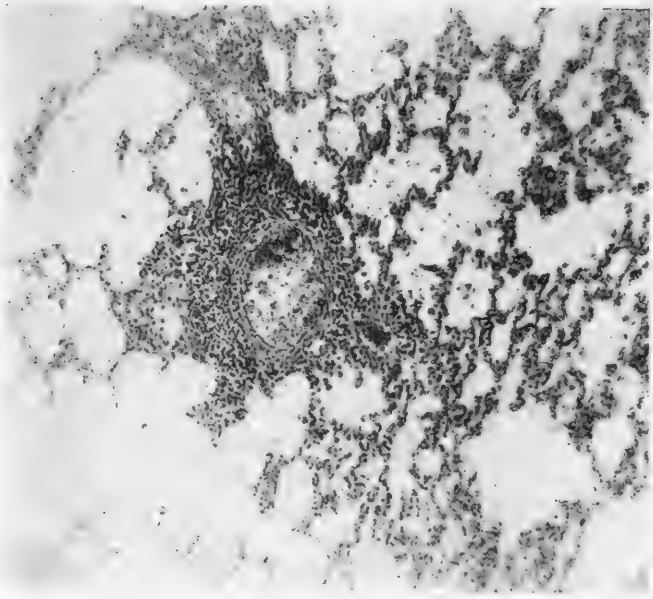

FIG. 16.

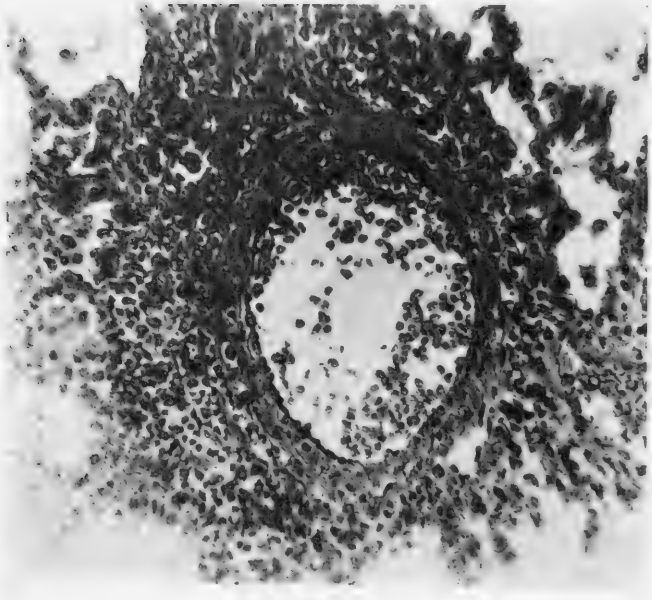

FIG. 17.

(Harris and Friedrichs: Periarteritis nodosa.)

STUDIES UPON EXPERIMENTAL MEASLES.

II. The Enanthematous, Exanthematous, Pyrexial, and Leucocytic Syndrome Produced in the Rabbit by Intravenous Inoculation of Blood from Cases of Human Measles.

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(Received for publication, February 27, 1922.)

In a previous paper[1] we reported the successful propagation of the virus of measles in the guinea pig, the criteria of transmission being the constant occurrence of leucopenia, pyrexia, and nephritis following, after a definite incubation period, the intracardiac injection of blood from cases of human measles. For this host, however, no well defined rash, or exanthem, was noted. In the absence of any definite cutaneous reaction, the symptoms observed, although striking, did not permit of the conclusion that the identical clinical picture of the human infection had been reproduced. Therefore, the purpose of the present series of experiments was not only to determine whether the virus of measles could be propagated in the rabbit by direct transfer of infected blood from man, but also to ascertain whether in this animal the experimental infection would induce the characteristic syndrome of the human disease.

While we were engaged upon the experiments herein reported there appeared the observations of Nevin and Bittman[2] upon the effect of the virus of measles on the rabbit. These authors record the appearance of enanthem, exanthem, and pyrexia in a great proportion of rabbits infected by the intracirculatory injection of

[1] Duval, C. W., and D'Aunoy, R., Studies upon experimental measles. I. The effects of the virus of measles upon the guinea pig, *J. Exp. Med.*, 1922, xxxv, 257.

[2] Nevin, M., and Bittman, F. R., Experimental measles in rabbits and monkeys, *J. Infect. Dis.*, 1921, xxix, 429.

citrated blood from cases of human measles. Hardie[3] also reports similar observations in which the rash appeared upon the shaved skin of rabbits on an average of 36 hours after the circulatory introduction of citrated blood from cases of measles.

EXPERIMENTAL.

Defibrinated human blood obtained from measles cases at the stage of temperature height was used for inoculation. The interval between collection of infected material and animal inoculation was never more than 1 hour, and during this intervening period the defibrinated blood was kept at a temperature of 37.5°C. Injections in rabbits were made into the marginal ear vein in a majority of cases, although in a few instances the intracirculatory introduction of infected material was accomplished by cardiopuncture.

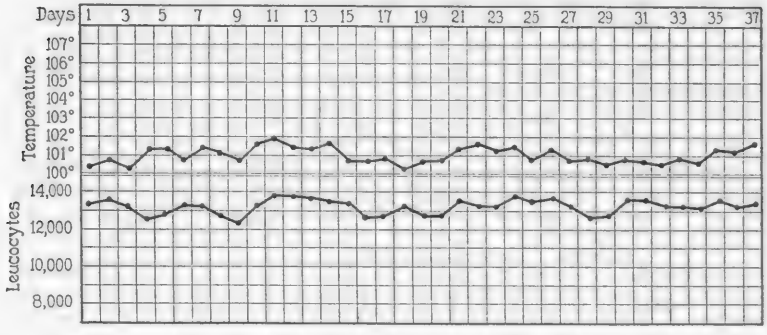
Experiment 1 (*Preliminary*).—The divergence of opinion regarding the normal variations in temperature and number of circulating leucocytes in rabbits made it necessary for us to determine these factors in order to establish a basis for the experiments. Twelve healthy animals were isolated, and their rectal temperatures and leucocytic counts determined daily for a period of 37 days. Text-fig. 1 represents the normal variations observed.

The results show that the average temperature of the rabbits was 101°F. In the experimental animal of this species we regarded as pyrexia only the reading about 103°F. The white elements averaged 13,000. Leucocytic counts below 9,000 are considered as evidence of leucopenia.

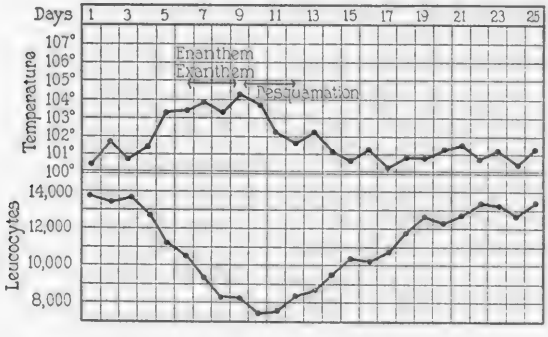
Experiment 2.—Here the purpose was to determine whether the virus of human measles could be directly carried from man to rabbit, and could induce in this host the clinical picture of the human infection. Blood from a typical case of human measles in the eruptive stage of the disease was employed as the inoculation material. Twenty rabbits comprised the number used in the experiment, sixteen being inoculated and four held as controls. The inoculated animals each received 2 cc. of human defibrinated virus blood injected into the marginal ear vein. Of the control animals two were isolated and injected intravenously with 3 cc. of normal human blood collected under aseptic conditions; the remaining two were not injected but left in the cage with the inoculated animals with the view of determining whether the infection

[3] Hardie, M., Essais de transmission expérimentale de la rougeole au lapin. Constatation d'un erythème sur la peau rasée, *Compt. rend. Soc. biol.*, 1921, lxxxiv, 968.

could be transmitted through contact. Cultures made from the blood containing virus before inoculation remained sterile for ordinary bacteria. Of the sixteen rabbits receiving the blood from cases of measles, seven showed, after a period of approximately 5 days, reactions indicative of specific infection. There was a sharp



TEXT-FIG. 1. Composite temperature and leucocytic charts of twelve normal rabbits.



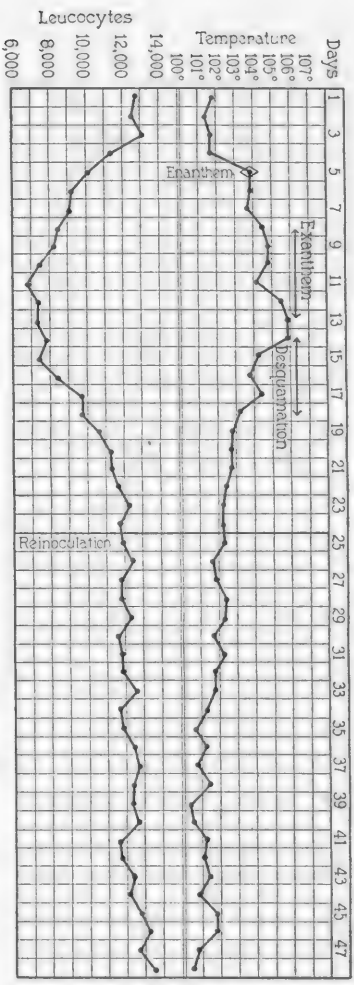
TEXT-FIG. 2. Typical reaction in the rabbit to blood containing the virus of measles.

rise in temperature of 2–3°F., beginning on or about the 5th day after inoculation in the majority of animals, with a coincident fall in the total number of circulating leucocytes. In some rabbits the rise in temperature did not appear before the 10th day. As a rule, the temperature remained high with slight variations for 2 to 4 days. During the pyrexial stage four animals developed an exanthem in the form of a dif-

fuse macular rash followed by the appearance of discrete papules over the neck and chest. At about the same time there were noted several sharply defined smaller erythematous patches on the buccal mucous membrane, resembling in the main the so called Koplik spots. The maculopapular eruption began to fade after 48 hours though it remained visible for 72 hours as a brownish discoloration. Branny or scaly desquamation invariably followed the rash. While only four of the seven reacting animals of this series presented a well defined rash, the leucocytic and temperature reactions in the remaining three were of sufficient significance to lead to the conclusion that the virus of human measles was capable of infecting the rabbit without giving rise to the cutaneous lesion. On the other hand, the appearance of an exanthem in the inoculated animal, coincident with the prominent and constant signs of pyrexia, leucopenia, coryza, etc., permits of the conclusion that the rabbit affords an experimental host in which the virus of measles produces the same clinical picture as observed in man. Text-fig. 2 graphically represents the typical reaction induced in the rabbit.

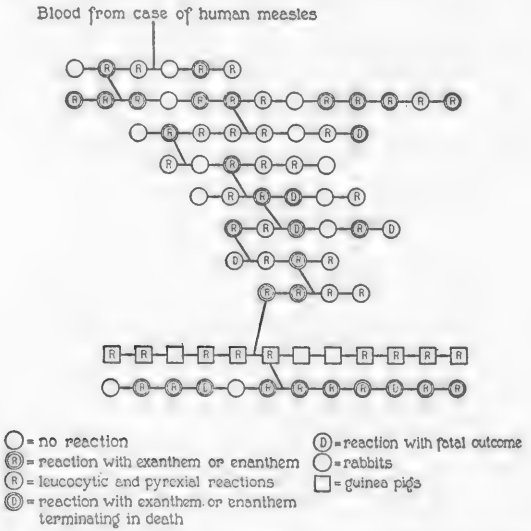
Experiment 3.—In order to determine the effects of reinoculation upon previously reacting animals, four rabbits of Experiment 2 which had shown typical exanthemata, leucocytic and pyrexial reactions were reinoculated, after complete recovery, with 2 cc. of blood from human and guinea pig measles cases. Two animals were used for the human virus, and two for the guinea pig virus, the blood containing the virus in both instances being obtained at the height of the reaction. In none of these animals were there noted any further changes in the temperature or cell count, nor the appearance of exanthemata. This would seem to prove that infection establishes an immunity (Text-fig. 3).

Experiment 4.—The purpose of this experiment was to continue the propagation of the virus by passage from rabbit to rabbit with the view also of increasing its virulence through repeated passage. Blood from animals reacting to the direct transfer of human measles virus was injected intravenously in 1 cc. quantities into twelve rabbits. In the animals of the second series the reaction was identical with that seen in the direct transfer animals. The proportion of reactions, however, was greater than with those of Experiment 2. Eleven of the rabbits reacted, nine of them showing exanthemata and enanthemata. After an incubation period of 3 to 5 days there occurred a rather abrupt rise in temperature of 1–4°F. and a coincident slight fall in the leucocytes. The temperature elevation continued usually for 24 to 36 hours in the majority of the animals; in the others it lasted with remission for 3 to 4 days. In the latter the leucocytes always showed the greatest decrease (well marked leucopenia). Furthermore, it may be said that the exanthem occurred in the animals with the more pronounced symptoms. In all, eight transfers from rabbit to rabbit were carried out, thus transmitting the virus through the eighth generation. In this experiment it is noteworthy that a number of rabbits died presumably as result of the virus, death occurring more frequently in animals of the later transfers. Such animals were visibly sick during the period of maximum reaction. Autopsies revealed grave nephritic changes, not unlike those reported by us for the measles-infected



TEXT-FIG. 3. Effects of reinoculation, with blood containing the virus of measles, of a rabbit which had reacted to a previous injection.

guinea pig. The kidneys were swollen, cloudy, and often infiltrated with hemorrhages. No noteworthy lesions were demonstrable in other internal organs, other than that common for toxemia. In no instance was pneumonia observed, nor were the ordinary pathogenic bacteria ever isolated from the heart's blood or internal organs of these fatal cases. This is of unusual interest because in fatal cases of human measles, pneumonia and septicemia almost invariably occupy the clinical field at death. While mortality is attributed in the human to secondary invaders, these seem to play no part in the experimental disease for rabbits and guinea pigs, the virus being capable *per se* of destroying the host. Text-fig. 4 expresses graphically the results of this experiment.


Blood from case of human measles
○ = no reaction
Ⓡ = reaction with exanthem or enanthem
R = leucocytic and pyrexial reactions
Ⓓ = reaction with exanthem or enanthem terminating in death
D = reaction with fatal outcome
○ = rabbits
□ = guinea pigs

TEXT-FIG. 4. The continued reaction in the rabbit through successive generations, and the cross-reaction through the rabbit and guinea pig.

Experiment 5.—Since it was found that the rabbit as well as the guinea pig reacts in a characteristic manner to direct intravenous injections of human blood from cases of measles a series of experiments was carried out to determine what effects would be induced by transmission of the virus from rabbit to guinea pig and *vice versa.* Accordingly, one set of twelve guinea pigs received intracardially 1 cc. of fresh defibrinated rabbit blood obtained from an infected animal at the height of the reaction. Another set of twelve rabbits was inoculated intravenously with 1 cc. of fresh defibrinated blood secured from an infected guinea pig at the time of maximum pyrexia. Typical reactions were secured in 75 per cent of the cross-inoculated guinea pigs and in ten of the twelve rabbits.

DISCUSSION AND SUMMARY.

An active transmissible virus exists in the blood of measles patients during the eruptive stage of the disease. This virus produces in rabbits after intravenous injection a specific reaction analogous in all essential features to that of the human infection. Following a definite incubation period of from 2 to 5 days the animals infected show pyrexial, leucocytic, and cutaneous alterations. Fully 90 per cent of such inoculated rabbits react in a remarkable manner. The earliest constant symptom of the infection is a rise in temperature, which on the average occurs 4 days after inoculation and most probably marks the end of the incubation period. Concomitantly with this temperature rise there is a diminution in the total number of circulating leucocytes. This decrease in the number of white blood elements may be relative or may appear in the form of a well defined leucopenia. The most striking objective signs are the coryza, conjunctival injection, enanthemata, and exanthemata. The mucous membrane lesions are similar in their physical appearance to the so called Koplik spots seen in man. They occur on the buccal side of the oral cavity ranging in number from two to eight discrete hemorrhagic areas with paler centers. They appear as a rule coincidently with the temperature rise or shortly thereafter. The exanthematous lesions though occurring only in about 40 per cent of the infected animals complete the clinical syndrome in this particular experimental host. The rash may appear as early as the 3rd and as late as the 7th day after inoculation. In its early stage it is of the macular variety, appearing as a diffuse eruption which later develops into a more papular type of lesion. At this time the cutaneous manifestations appear as slightly raised, flattened, purplish red, discrete areas in the skin of the face, neck, chest, and abdomen.

Repeated passage of the virus of measles through the rabbit seems to increase its virulence. A number of animals infected with such passage virus succumb in the fourth and subsequent generations, undoubtedly as the direct result of the action of the specific excitant, as in none of the animals was there cultural evidence of secondary intercurrent infection. In the animals dying presumably as a result of the specific virus grave nephritic changes were evident. It is a

noteworthy fact that the pneumonia so common in fatal cases of human measles was not evident in any of the experimental animals. We believe this to be of considerable significance, especially in elucidating the direct etiological factor of the fatal pneumonias so often present in human measles cases. Apparently such infections in man can be explained purely on the basis of the destruction of normal defense barriers by the specific excitant of the infectious disease, and the lack of host resistance to the ordinary pyogenic microorganism.

STUDIES UPON EXPERIMENTAL MEASLES.

III. The Symptom-Complex in the Guinea Pig and Rabbit Following the Intratracheal and Intravenous Injections of Filtered Nasopharyngeal Secretions from Cases of Human Measles.

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In previous communications[1,2] it has been shown that guinea pigs and rabbits when injected intravenously with blood secured from cases of human measles at the height of the exanthem react specifically. This reaction consists in pyrexial changes, leucopenia, and in the great majority of animals enanthemata and exanthemata fairly characteristic of the human disease. In this study we desire to record the reaction produced in these animals as a result of the introduction into their circulatory and respiratory systems of filtered nasopharyngeal washings from cases of human measles.

LITERATURE.

That the nasopharyngeal secretions of patients suffering from measles contain the specific virus is at present unquestioned. Goldberger and Anderson[3] by means of filtered and unfiltered buccal and pharyngeal secretions from cases of human measles injected into the respiratory tract of monkeys produced a symptom-complex which

[1] Duval, C. W., and D'Aunoy, R., Studies upon experimental measles. I. The effects of the virus of measles upon the guinea pig, *J. Exp. Med.*, 1922, xxxv, 257.

[2] Duval, C. W., and D'Aunoy, R., Studies upon experimental measles. II. The enanthematous, exanthematous, pyrexial, and leucocytic syndrome produced in the rabbit by intravenous inoculation of blood from cases of human measles, *J. Exp. Med.* 1922, xxxvi, 231.

[3] Goldberger, J., and Anderson, J. F., The nature of the virus of measles, *J. Am. Med. Assn.*, 1911, lvii, 971.

they interpreted as mild manifestations of the disease. Blake and Trask[4] working with unfiltered nasopharyngeal secretions introduced intratracheally into *Macacus rhesus* confirmed and extended these early observations Grund[5] by intratracheal injections of filtered and unfiltered nasopharyngeal secretions, obtained from the human case during the preeruptive and early eruptive stages of the disease, succeeded in producing a series of reactions in rabbits which were considered suggestive in a great proportion of the experiments as manifestations of the human disease.

EXPERIMENTAL.

The nasopharyngeal secretions used for these experiments were secured from cases of the human disease at the height of the cutaneous reaction. In obtaining the secretions sterile applicators tipped with cotton were introduced through West tubes into the nares and around the tonsillar surfaces. The soiled applicators were then washed in small portions of 0.85 per cent sterile sodium chloride solution previously kept at a temperature of 37°C. In a large number of instances as many as twenty infected applicators were obtained from a single individual, but the amount of saline solution used in washing those from one case was never more than 10 cc. In several cases the bacterial flora of the washings was studied. *Streptococcus hæmolyticus* and *non-hæmolyticus*, *Streptococcus viridans*, *Bacillus coli communis*, *Bacillus influenzæ*, and *Staphylococcus aureus* were the only microorganisms cultivable by ordinary methods that were encountered. Filtration of washings was made through Berkfeld N filters, the filtrate in all instances showing no ordinary bacteria. From the time of securing washings to the time of animal inoculations not more than 1 hour elapsed, and during this time the washings were maintained at a temperature of between 37.5° and 39°C. Intratracheal injections were always made under ether anesthesia. The trachea of the experimental animal was exposed, it was then steadied, and the material was slowly injected into the upper and lower part of the respiratory tract through a needle inserted between the cartilaginous rings. As much as 10 cc. of fluid could thus be injected

[4] Blake, F. G., and Trask, J. D., Jr., Studies on measles. I. Susceptibility of monkeys to the virus of measles, *J. Exp. Med.*, 1921, xxxiii, 385.

[5] Grund, M., Susceptibility of rabbits to the virus of measles. Inoculations with nasopharyngeal material, *J. Infect. Dis.*, 1922, xxx, 86.

in a full grown rabbit with no subsequent discomfort to the animal. A single catgut suture was used to approximate the margin of the skin incision. In rabbits the material was injected into the marginal ear vein and in guinea pigs it was administered by cardiopuncture.

Experiment 1.—A number of rabbits (Table I) were injected directly into the trachea with 10 cc. of pooled filtered nasopharyngeal washings. At the same time a series of control animals was similarly injected with filtered washings secured from normal nasopharynges and from patients suffering with lobular pneumonia. Rectal temperature determinations, leucocytic counts, and observation for enanthemata and exanthemata were performed at stated intervals thereafter. All of the animals of the series receiving filtrates obtained from measles cases reacted similarly in as far as pyrexia and leucocytic depression were concerned. After an incubation period varying from 3 to 7 days there occurred an elevation of temperature amounting to as much as 3° in a number of cases.[6] Coincident with the beginning of temperature rise, or shortly thereafter, enanthemata were noted in approximately 90 per cent of the animals. These appeared on the buccal and hard palate mucosæ as minute anemic areas surrounded by a pinkish to reddish zone. Exanthemata occurred in 60 per cent of the animals, appearing about the time of temperature height (5 to 9 days). The rash usually appeared first about the face, varying from a diffuse macular reaction to well marked papules. At the height of the cutaneous reaction well defined papules could be observed over the neck between the front quarters, and upon the abdominal wall of the animal. Invariably after the disappearance of the rash, branny or scaly desquamation took place. All the animals manifesting an exanthem appeared sick, and of these a number subsequently died (Table I). Conjunctival injection was always a prominent sign. In most of the animals no well marked leucopenia was noted, though a relative fall in the leucocytes always occurred, but this within wide limits. The control animals inoculated with filtered washings from other individuals failed to react in any manner. Text-fig. 1 expresses graphically the findings in a typical reacting animal of the series.

Experiment 2.—The susceptibility of the rabbit to the intracirculatory introduction of filtered nasopharyngeal washings was here tested. 5 cc. of filtrate obtained as above outlined were injected into the circulation of a number of full grown rabbits (Table I). These were observed over a period of 4 weeks, leucocytic and temperature curves being drawn for all animals. Of the series injected, 80 per cent showed unmistakable evidences of a reaction, which reaction we interpret as ascribable to the effects of the transmitted virus of measles. Again, in these series well marked leucopenia was not noted for all animals, but enanthem, exanthem, and temperature reactions were regularly encountered.

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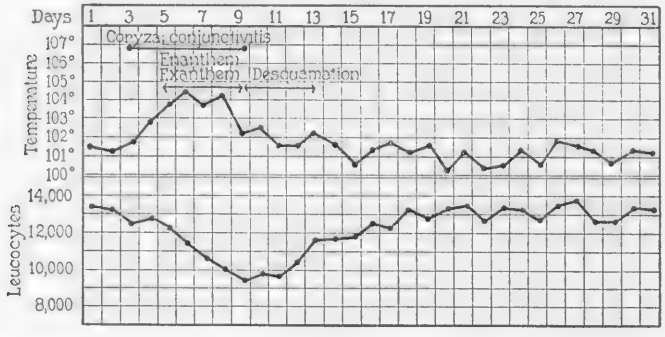
[6] As in the previous study we consider only temperatures of 102°F. or over as pyrexia.

Experiment 3.—Intratracheal injections of 2 cc. portions of filtered washings were made in a number of guinea pigs (Table I). Of the animals thus injected, 75 per cent reacted in a manner identical to that previously reported by us as characteristic when blood from cases of human measles was injected into the guinea pig. Regularly in the reacting animals 9 to 14 days after injection, the temperature rose with a coincident fall of the circulating leucocytes. 10 per cent of the injected animals succumbed with no gross lesions discernible at necropsy, excepting an acute hemorrhagic nephritis. Animals dying failed to show in their blood stream or in their organs by ordinary cultural methods any microorganisms which could be considered responsible for death. Typical enanthemata and exanthemata were not observed in any of the guinea pigs of the series; however, in a number a few discrete hemorrhagic areas were noted on the buccal mucous membranes before the appearance



TEXT-FIG. 1. Reaction in the rabbit following intratracheal injection of filtered nasopharyngeal material from cases of human measles.

of the temperature rise, but as these were not grossly characteristic as compared to the Koplik spots seen in man or the lesions noted in the rabbit after experimental transmission of the virus of measles, they are not stressed in the present report. Exanthemata are either evanescent, difficult of recognition, or do not occur in the guinea pig, as we failed to note such cutaneous manifestations in any of the animals. Attempts at removing the hair from the abdomen of normal guinea pigs, either by shaving, clipping, or cutting with scissors, caused confusing erythemata of from 2 to 4 days duration.

Experiment 4.—2 cc. portions of filtered washings were introduced into the circulation of several guinea pigs of average weight of 250 gm. 90 per cent of the animals thus injected reacted typically after incubation periods varying from 7 to 11 days (Table I). Well marked leucopenia was observed in all the reacting animals of the series. Subcultures from blood obtained from the reacting animals of this series

when injected into rabbits gave rise after definite incubation periods to typical reactions characterized by temperature changes, exanthemata, and enanthemata.

Experiment 5.—Five guinea pigs and five rabbits that had reacted characteristically after the intratracheal injection of filtered washings and had completely recovered, were given intracirculatory injections of defibrinated blood secured from cases of human measles at the height of eruption. None of these animals reacted in any manner. This is in keeping with previous observations upon the immunity in the guinea pig conferred by the intracirculatory injection of blood from cases of human measles.

TABLE I.

Reaction in Rabbits and Guinea Pigs Following Inoculation with Nasopharyngeal Material from Cases of Human Measles.

Experimental animal.	No. of animals inoculated.	Method of inoculation.	No. of animals reacting.						Mortality.
			Enanthem.	Exanthem.	Conjunctivitis and coryza.	Desquamation.	Leucopenia.	Pyrexia.	
Rabbit.	12	Intratracheal.	10	7	10	7	Marked, 4. Relative, 5.	11	2
"	10	Intracirculatory.	8	8	8	8	Marked, 2. Relative, 6.	9	1
Guinea pig.	12	Intratracheal.	3	0	0	0	Marked, 9.	11	2
" "	10	Intracirculatory.	4	0	0	0	" 9.	10	3

DISCUSSION.

As a result of the experiments above recorded it seems conclusively established that the intratracheal and intracirculatory introductions in guinea pigs and rabbits of filtered nasopharyngeal secretions from cases of human measles occasion a definite and constant reaction. This reaction is characterized by a complex of objective and subjective signs which very closely resemble the manifestations in man of the acute infectious disease measles. In the guinea pig the incubation period following injection ranges between 9 and 14 days, approximately the same length of time previously noted by us following the intracirculatory introduction in this animal of defibrinated blood from cases of measles. Leucopenia is always a constant change, appearing about the time of temperature rise or shortly before, and lasting throughout the period of pyrexia. Enanthemata, never quite characteristic grossly as compared to Koplik's spots in

man, are fairly constant, appearing at the period of pretemperature or early temperature rise. Histologically, however, these enanthematous lesions present the identical changes described by Mallory and Medlar[7] in human Koplik spots. Exanthemata were never definitely noted. We believe that they are difficult to elicit in the guinea pig. We were often confused by the peculiar erythema appearing in normal guinea pigs after the removal, by shaving or cutting, of hair. This non-specific macular rash persisted in a number of instances for from 3 to 4 days, being followed by a light desquamation. Conjunctival irritation is always prominent in reacting animals. Repeated aerobic and anaerobic cultures from the conjunctival sacs of such animals failed to reveal organisms deserving any special mention.

In the rabbit, reaction always manifests itself early, on an average in 4 days. the extreme limits noted in our series being 2 and 7 days. Conjunctival injection and enanthemata are generally the early evidences of the symptom-complex. The buccal mucosal lesions appear first as anemic areas surrounded by an injected areola, and generally develop into distinct papules. As many as eight such papules can be noted in some of the animals. At the time of enanthem appearance or shortly thereafter a sharp rise of temperature takes place, amounting to as much as 4° in most cases. With the appearance of pyrexia, the cutaneous reaction manifests itself. As a rule, the exanthem appears first about the face in the form of a diffuse macular eruption. Soon afterward a similar rash appears on the neck, between the front quarters, and on the abdominal wall. Typical papules now develop, which are present for from 1 to 3 days. With the onset of papules there is usually a fall in temperature, with a gradual return to normal. Desquamation, usually branny, sometimes scaly, appears regularly. There is always a relative drop in the circulating leucocytes, at the expense of the neutrophilic element, without, however, the very sharp decline noted in the guinea pig.

The regularity with which it has been possible to produce this symptom-complex in guinea pigs and rabbits following the injection of filtered nasopharyngeal washings from cases of human measles and the similarity of the symptom-complex to the manifestations

[7] Mallory, F. B., and Medlar, E. M., The skin lesion in measles, *J. Med. Research*, 1919–20, xli, 327.

of measles both in man and in the guinea pig and rabbit following the intracirculatory injection of blood from cases of human measles, lead us to agree with the conclusion that there is present in the nasopharyngeal secretion of measles cases as a causal agent of the disease a filter-passing virus.

We believe that experimental measles, at least in the rabbit and guinea pig, is primarily an infection of the blood, and that the mucous membrane lesions are secondary. It seems necessary that the virus should enter and propagate in the blood stream before any manifestations of the infection present themselves. The coryza, conjunctivitis, and other early signs are not to be regarded as indications of a primary mucous membrane localization of the specific excitant, but are the earliest outward evidences of an already established blood infection. The course of the experimental infection in these animals varies but little whether the infective material is nasopharyngeal secretions or blood, and whether the inoculating route is intravenous or intratracheal. This is not surprising when we consider that practically instantaneous absorption[8] of intratracheally deposited material takes place, naturally with rapid entrance into the circulation. If the localization of the specific incitant took place in the mucous membranes by the selective action of this incitant, it would seem plausible to expect an earlier manifestation of both objective and subjective signs following the direct infection of mucosal surfaces than following infection by the intracirculatory route.

CONCLUSIONS.

1. There is present as a causal agent in the nasopharyngeal secretions of measles cases a filter-passing virus.

2. The rabbit and the guinea pig react specifically to the intratracheal and intracirculatory injections of filtered nasopharyngeal secretions secured from cases of human measles.

3. Enanthem, exanthem, and pyrexial disturbances characterize this specific reaction in the rabbit; in the guinea pig the reaction manifests itself by pyrexia, marked leucopenia, and grave nephritis in the fatal cases.

[8] D'Aunoy, R., Antibody production after intratracheal injection of antigen, *J. Infect. Dis.*, 1922, xxx, 347.

CHANGES IN THE NUMBER OF SMALL LYMPHOCYTES OF THE BLOOD FOLLOWING LIGATION OF THE THORACIC DUCT.

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INTRODUCTION.

The important work of Murphy and his collaborators has added great interest to the study of the physiology of the lymphocyte. These investigators have shown particularly well the rôle that the lymphocyte plays in the resistance and susceptibility of mice to cancer grafts. Previously the lymphocyte was studied considerably in regard to the extent of its appearance in the thoracic duct under ordinary conditions or its appearance in the blood stream following splenectomy (Biedl and von Decastello), after extirpation of groups of important glands (Ehrlich and Reinbach), after the administration of pilocarpine (Rous), and following ligation of the thoracic duct (Davis and Carlson; Bunting and Huston).

During the progress of work on the ligation of the thoracic duct recently reported (Lee), the opportunity offered itself for studying the effect of the ligation of that vessel on the number of small lymphocytes in the blood.[1]

Materials and Methods.

Young adult male cats were used for all of the experimental work. From blood which was procured from the ear of the animal, a white blood count and a smear were obtained. Wright's stain was applied to the smear and a differential count of 300 cells or more was made. Two control counts were made; one was obtained on the day preced-

[1] All operations were performed under ether anesthesia.

ing operation, and the other was taken 24 hours later, on the morning of the day of operation. The care of the animal before, during, and after the operation was the same as reported previously; likewise, the operation, which was essentially an intrathoracic ligation of the thoracic duct and the periaortic lymphatic plexus, was similar. 5 hours after operation the blood was examined again. On the following day counts were made in the morning and afternoon, while from then on the examinations were made in the morning of the next 7 successive days, and after that on alternate days. At the end of about 3 weeks the lymphocytes were again up to the preoperative level, and the animal was then sacrificed after the mesenteric lymphatics had been injected with Berlin blue. Blocks of tissue from the various organs were fixed in formaldehyde for further study, and a dissection of the collateral circulation of the thoracic duct was made.

Since the data from the entire series of five animals are about the same, it is only necessary to report the findings in two animals in order to illustrate the changes in the number of small lymphocytes in the circulating blood.

In the differential counts here reported the white blood cells have been classified under the following headings: polymorphonuclear neutrophils, eosinophils, basophils, small lymphocytes, large lymphocytes, large mononuclears, and transitionals. The polymorphonuclear neutrophils need no discussion. The eosinophils were not constant with respect to their granulation; the size of the granules was constant for any particular cell, though it varied for the different cells. Busch and Van Bergen have classified eosinophils into three groups according as the granulation was fine, medium, or coarse; yet it is obvious that such a classification is difficult. In all the smears that were examined a typical basophil was not seen. Very infrequently a cell was seen which had an irregularly shaped nucleus with a slightly granular purplish red cytoplasm; but the granulation was not typical of a basophil.

The small lymphocyte was taken to be a cell smaller than the polymorphonuclear neutrophil, round, with a round nucleus which was sometimes slightly indented. The nucleus was clearly outlined, and occupied almost the entire cell; its chromatin was dense, often aggregated, and deeply purple-stained. The cytoplasm was scant,

slightly blue-stained as a rule, with a small colorless rim around the nucleus, and it frequently contained a few fine azurophil granules.

The large lymphocyte differed from the small lymphocyte mainly in size, since the staining was essentially the same; there is a possibility, then, of considering these two cells as belonging to the same class. The nucleus of the large lymphocyte was larger than that of the small lymphocyte, the cytoplasm surrounded the nucleus definitely, and the azurophilic granules were larger and more numerous.

The large mononuclear was the largest blood cell observed in the cat; it was often several times as large as the polymorphonuclear neutrophil. The nucleus was large, often oblong, sometimes slightly indented; the chromatin was loose and skein-like and did not stain heavily. The cytoplasm was abundant, usually slightly granular or mottled, and contained, as a rule, numerous fine azurophil granules. It was often difficult to distinguish between this cell and the large lymphocytes, and undoubtedly the tables herewith submitted suffer because of this difficulty.

The transitional cell was considered to be similar to the large mononuclear and different mainly in that it had a markedly indented nucleus.

The recent work of Sabin has shown how vitally stainable granules may serve as a specific criterion for the differentiation of the three strains of the white blood cells of the chick; and there is reason to believe that this method may be applied generally to all blood examinations and can thus supplement the routine differential blood counts. Simpson has described how the vital dyes may serve to differentiate large mononuclear cells from transitional cells. Thorne and Evans also have employed vital dyes in showing the very low monocyte count of lymph in rabbits.

EXPERIMENTAL DATA.

With these standards for the identification of the cells in the differential counts made with Wright's stain, the experimental observations were carried out as described. The general technical procedures are indicated in the protocol of Cat 1, which is given below in condensed form.

Cat 1.—May 9, 1921. Young adult male cat, black; in stock since May 6, 1921. 10 a.m. Count made.

May 10. Food withheld for the last 24 hours. Weight 3,135 gm. 10 a.m. Count made. 11 a.m. Ligation of thoracic duct according to method already described. 5 p.m. Count made.

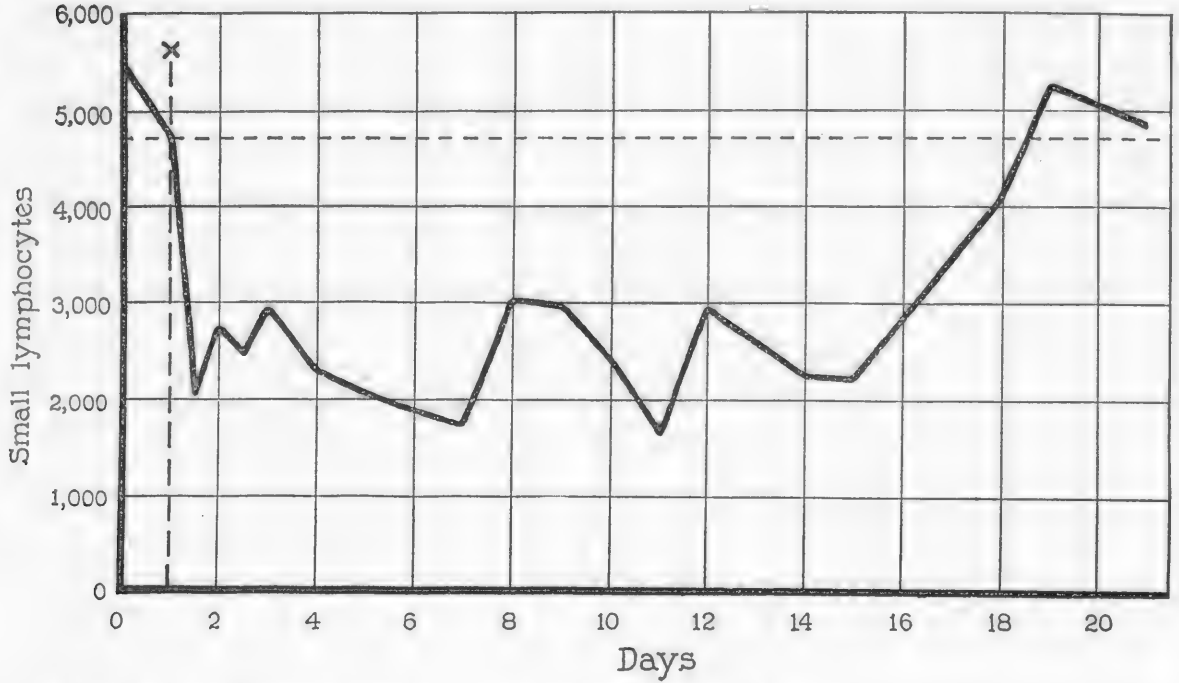
TABLE I.

Cat 1.

Date.	Total white blood count.	Polymorphonuclear neutrophils.	Eosinophils.	Small lymphocytes.	Large lymphocytes.	Large mononuclears.	Transitionals.	Absolute No. of small lymphocytes.
1921		*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	
May 9	15,720	51.3	11.0	35.0	1.0	1.3	0.3	5,502
" 10, a.m.	13,880	50.3	11.7	34.0	1.3	1.7	1.0	4,689
Operation.								
May 10, p.m.	30,600	88.3	0.7	6.7	2.7	1.0	0.7	2,050
" 11, a.m.	23,640	83.3	0.7	11.7	2.0	1.7	0.7	2,765
" 11, p.m.	22,800	84.0	1.0	10.7	2.0	1.3	1.0	2,439
" 12	19,900	75.7	4.3	14.7	2.7	1.7	1.0	2,925
" 13	14,280	70.7	10.7	16.3	1.3	0.7	0.3	2,327
" 14	13,880	71.3	11.7	15.0	0.7	1.0	0.3	2,080
" 16	13,760	73.7	9.0	12.3	4.0	0.7	0.3	1,692
" 17	17,400	70.7	4.3	17.3	3.7	3.3	0.7	3,010
" 18	13,800	66.7	6.0	21.3	3.3	1.7	1.0	2,939
" 19	13,600	68.7	6.3	17.7	3.0	3.3	1.0	2,407
" 20	10,560	71.7	7.3	15.7	1.0	3.3	1.0	1,657
" 21	12,000	65.3	7.0	24.7	1.0	1.3	0.7	2,964
" 23	12,520	70.7	8.7	18.3	0.7	1.0	0.7	2,291
" 24	10,800	71.3	6.3	20.3	1.0	0.7	0.3	2,192
" 27	16,640	60.7	10.0	24.7	3.3	1.0	0.3	4,109
" 28	18,000	56.3	8.7	29.0	2.7	2.7	0.7	5,220
" 30	17,440	60.7	9.7	27.3	1.3	0.7	0.3	4,861

Cat made uneventful recovery; the incision healed perfectly. Counts made are listed in Table I, and shown in Text-fig. 1.

May 30. Weight 3,420 gm. Since counts showed that the number of small lymphocytes in the circulating blood had reached the same level as before operation, the animal was sacrificed; the mesenteric lymphatic vessels were injected as described, and it was found that a lymphatovenous communication had established itself between the thoracic duct and the ninth left intercostal vein. The specimen was fixed in formaldehyde.



In all the figures the horizontal broken line indicates the level at the time of operation.

TEXT-FIG. 1. Curve to show the absolute number of small lymphocytes per cubic millimeter of blood in Cat 1 in which the thoracic duct was ligated at X. On the 19th day the number of small lymphocytes had again reached its preligation level.

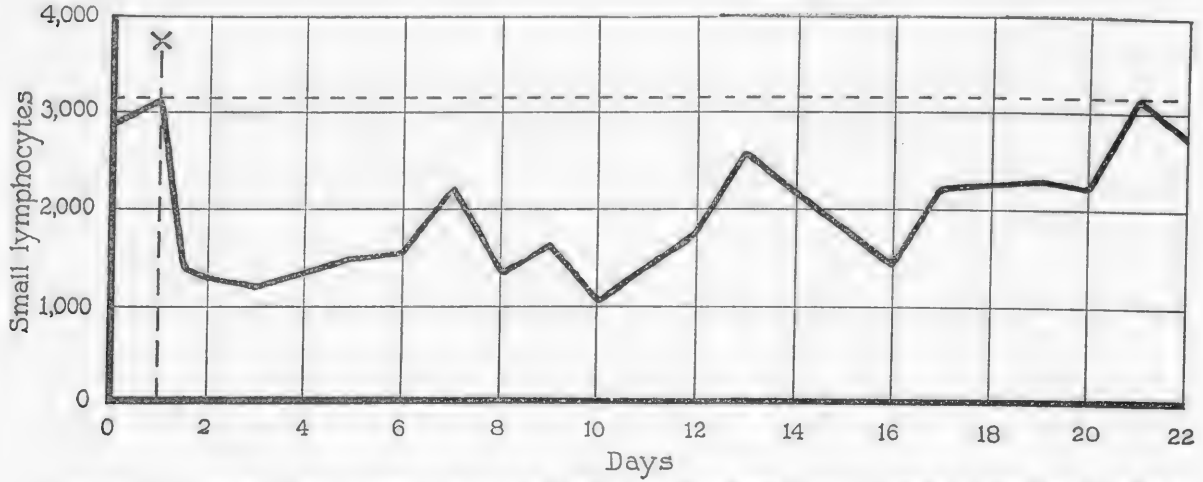
In this animal (No. 1) the number of small lymphocytes decreased 56 per cent immediately following the ligation of the thoracic duct. This reduction in the number of lymphocytes in the circulating blood endured until the preligation level was attained on the 21st day.

TABLE II.

Cat 2.

Date.	Total white blood count.	Polymorphonuclear neutrophils.	Eosinophils.	Small lymphocytes.	Large lymphocytes.	Large mononuclears.	Transitionals.	Absolute No. of small lymphocytes.
1921		*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	
May 25	13,400	71.0	4.7	21.0	1.3	1.0	1.0	2,814
" 26, a.m.	13,080	69.3	4.7	24.0	1.0	0.7	0.3	3,139
				Operation.				
May 26, p.m.	38,160	94.0	0.8	3.6	0.6	0.8	0.2	1,373
" 27, a.m.	19,240	90.7	0.3	6.7	1.3	0.7	0.3	1,288
" 28	20,760	92.0	1.0	5.7	2.0	1.0	0.3	1,183
" 30	26,360	90.0	0.7	5.7	1.3	1.0	1.3	1,482
" 31	22,320	86.3	3.0	7.0	1.3	2.0	0.3	1,562
June 1	20,600	79.7	5.0	11.0	2.7	1.3	0.3	2,266
" 2	16,800	84.3	4.0	7.7	2.7	1.0	0.3	1,293
" 3	18,000	86.3	2.3	9.0	2.0	0.3	0	1,620
" 4	21,280	90.0	2.0	5.0	1.3	1.3	0.3	1,064
" 6	20,840	82.7	5.3	8.3	1.7	1.0	1.0	1,729
" 7	19,400	77.0	6.0	13.3	2.3	1.0	0.3	2,580
" 8	19,120	80.7	4.7	11.3	1.3	1.0	1.0	2,160
" 10	17,000	83.7	5.0	8.3	1.0	1.3	0.7	1,411
" 11	16,800	77.0	5.7	13.3	1.3	1.3	1.3	2,234
" 12	11,480	72.3	3.3	20.0	1.7	1.7	1.0	2,296
" 14	12,960	72.7	6.3	17.3	2.0	1.3	0.3	2,242
" 15	14,640	69.0	3.7	21.3	4.3	1.3	0.3	3,118
" 16	18,400	80.3	2.0	14.7	1.3	1.3	0.3	2,704

At this time an anatomical pathway capable of affording ingress of cells of the thoracic duct lymph into the systemic blood stream was demonstrated as a lymphatovenous communication between the thoracic duct and the ninth intercostal vein. The experiment was free from complications; absence of infection served to render the blood counts dependable.



TEXT-FIG. 2. Curve to show the absolute number of small lymphocytes per cubic millimeter of blood in Cat 2 in which the thoracic duct was ligated at X. On the 21st day the number of small lymphocytes had reached its preligation level.

A somewhat similar condition was shown by Cat 2, which weighed 3,030 gm. before operation, and 3,000 gm. when sacrificed. In this case, which likewise was free from concurrent infection, the lymphatovenous connection was between the thoracic duct and the azygos

TABLE III.

Cat 3.

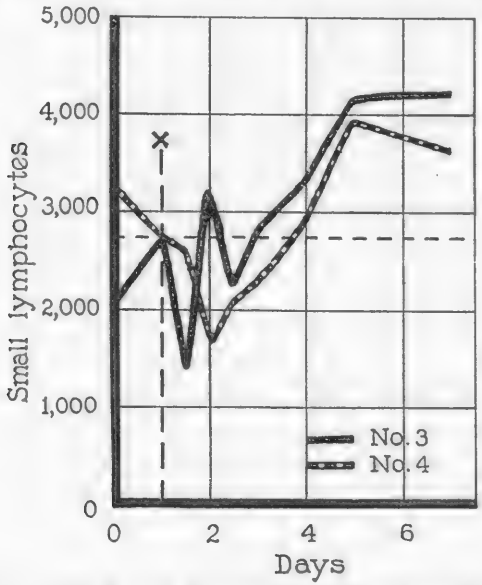
Date.	Total white blood count.	Polymorphonuclear neutrophils.	Eosinophils.	Small lymphocytes.	Large lymphocytes.	Large mononuclears.	Transitionals.	Absolute No. of small lymphocytes.
1921		*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	
Apr. 25	15,920	78.0	3.3	12.7	2.7	1.3	2.0	2,021
" 26, a.m.	14,840	71.0	6.0	18.3	3.0	1.0	0.7	2,715
Operation.								
Apr. 26, p.m.	18,040	88.0	0.3	7.7	1.3	2.0	0.7	1,389
" 27, a.m.	15,680	71.3	3.7	20.7	2.3	1.3	0.7	3,245
" 28	14,120	70.0	2.0	20.0	5.3	1.7	1.0	2,824
" 29	15,920	73.7	2.3	21.0	1.3	0.7	1.0	3,343
" 30	12,320	59.3	3.3	34.0	1.7	0.7	1.0	4,188
May 2	12,960	63.3	1.7	32.7	1.3	0.7	0.3	4,237
Cat 4.								
Apr. 25	8,480	54.7	3.0	38.3	2.0	1.3	0.7	3,247
" 26, a.m.	8,360	61.0	3.0	32.7	2.0	1.0	0.3	2,733
Operation.								
Apr. 26, p.m.	17,640	79.7	0.7	14.7	3.0	1.3	0.7	2,592
" 27, a.m.	9,680	77.3	1.7	16.7	1.7	2.0	0.7	1,616
" 27, p.m.	9,840	75.7	1.3	21.0	1.0	0.7	0.3	2,066
" 28	7,560	61.7	5.0	30.0	1.7	1.3	0.3	2,268
" 29	9,200	58.3	7.3	32.0	1.3	0.7	0.3	2,944
" 30	11,920	57.3	7.0	33.3	1.0	1.0	0.3	3,969
May 2	11,280	58.3	5.0	32.0	1.7	1.7	1.3	3,609

vein at the ninth interspace. The number of lymphocytes in the circulating blood also decreased by 56 per cent immediately following ligation, but gradually returned to the preligation level on the 19th day. Table II and Text-fig. 2 show the counts in this animal.

In order to check up the findings reported above, two control experiments were made. These animals were treated identically like

the ones above mentioned, with the exception that instead of performing the actual ligation in the chest, the operative procedure was limited to a slight dissection of the anterior periaortic tissue. The animals made a good recovery, and their blood counts are listed in Table III, and demonstrated in Text-fig. 3.

From the data herewith submitted it is seen that the intrathoracic ligation of the thoracic duct caused an immediate fall of about 56



TEXT-FIG. 3. Curve to show the absolute number of small lymphocytes per cubic millimeter of blood in Cats 3 and 4 which were used as controls. On the 4th day the number of small lymphocytes had already reached its preoperative level.

per cent in the number of small lymphocytes in the circulating blood, and that the number gradually returned to the preoperative level about 3 weeks after ligation (Text-figs. 1 and 2). In the one case the lymphatovenous connection was with the ninth left intercostal vein; and in the other the connection was with the azygos vein directly. It seems then that at the end of 21 days these lymphatovenous connec-

tions were of sufficient size to perform the functions previously exercised by the thoracic duct. Whether one can use the number of small lymphocytes in the blood as an absolute criterion for the degree of establishment of the collateral circulation is still questionable; nevertheless, the counting of the small lymphocytes is one of the more obvious and accessible methods for attacking this problem.

It will be noted that the decrease in the number of lymphocytes in the blood as shown by Tables I and II was invariably around 56 per cent; in the entire series this decrease has been between 54 and 64 per cent. This figure is somewhat lower than that of the only experiment in the series in which the ligation of both thoracic ducts alone was performed (No. 10, Biedl and von Decastello) in which a decrease of 79 per cent was obtained. In this case the thoracic duct on the left side, as well as all visible lymphatic vessels in the right side of the neck, was ligated. It is obvious that such a procedure shut off more lymphatic vessels than a simple ligation of the thoracic duct in the chest, and consequently one would expect a greater decrease in the number of lymphocytes in the circulating blood. For if the thoracic duct is obstructed in the chest, then the lymph of the upper extremities, head, neck, and upper part of the thorax, may still enter the blood stream through normal channels and thus furnish a considerable number of lymphocytes to the blood stream.

The control experiments showed that although there was also a decrease in the number of small lymphocytes when the duct had not been tied, yet this decrease was only temporary, for the preoperative level was exceeded on the 2nd or 3rd day (Text-fig. 3). Other cats which simply received an ether anesthetic for a half hour showed a temporary fall in the number of small lymphocytes.

DISCUSSION.

Many investigators have studied the thoracic duct with respect to the number and kind of cells for which it forms the pathway to the blood stream. Winternitz in 1895 concluded that the interruption of the lymph flow into the blood *via* the thoracic duct does not prevent leucocytosis. In 1901, Biedl and von Decastello described changes in the blood picture of dogs in which either a fistula of the duct was made with or without splenectomy, or in which the duct was tied under aseptic technique. They found that the mononuclears, including the small

lymphocytes, decreased in the blood from 62 to 79 per cent in absolute numbers. They recorded only two experiments, Nos. 9 and 10, in which they ligated the duct and studied the blood. In Experiment 9 ligation of the duct produced practically no change in number of mononuclears in the blood; this condition was accounted for by the demonstration of a branch of the thoracic duct below the point of ligation which connected with veins on the right side of the neck. In the other experiment, No. 10, the thoracic duct as well as all lymphatics on both sides of the neck were ligated, and it was found that the number of mononuclears in the blood diminished 79 per cent on the day following ligation, but on the 4th day after ligation the decrease was only 29 per cent. Blood counts were made until 1 week after operation. No attempt was made to determine whether the mononuclear cells in the blood would come back to the preligation level. Furthermore, the animal in Experiment 10 had an infection of the operative wounds; though the infection cleared up, nevertheless the inflammatory process undoubtedly brought in an unwelcomed factor which must have influenced the blood examination. The authors concluded that ligation of the thoracic duct or production of a fistula of the same did not entirely prevent the entrance of mononuclears into the blood stream; nor did injections of a colored solution serve to demonstrate connections between the lymph and blood systems. However, more recent work has shown that collateral paths may become established after the thoracic duct has been ligated.

In 1904 Selinoff reported experiments on dogs in which he cannulated the thoracic duct under aseptic precautions and studied the resultant change in the number of cells in the blood stream. He divided the white cells into three groups, young, adult, and old. This division corresponds essentially to lymphocytes, monocytes, and polymorphonuclears, respectively. In one experiment, No. 18, the lymphocytes dropped from 1,600 before operation to 20 on the 14th day after operation. However, the remarkable decrease in lymphocytes was attended also by a decided increase in the number of polymorphonuclears; thus the animal in the experiment cited above had on the 14th day polymorphonuclears present in the blood to the extent of 99.7 per cent. Not only did this experiment show a high polymorphonuclear content, but other experiments which were extended only to the 5th day after operation, such as No. 12, showed polymorphonuclears in the blood to the extent of 98.0 per cent. Obviously infection was present in all of the operative wounds of these experimental animals because of the thoracic duct fistula. It would seem then that in these cases the infection was characterized not only by a great increase in the number of polymorphonuclear leucocytes but also by an astonishing decrease in the number of lymphocytes in the circulating blood.

Also in 1904 Banti, in reporting the work done in his laboratory by Crescenzi, stated that after splenectomy and subsequent establishment of a thoracic duct fistula in dogs the lymphocytes of the blood decreased from one-half to ten-elevenths, but returned to normal or above normal at the end of 4 days, at which

time, it was claimed, there did not exist any accessory lymphatic channels between the thoracic duct and the subclavian vein. He was at a loss to account for the manner in which the lymphocytes reached the blood stream and considered as a probability the entrance of lymphocytes directly into the blood stream while still in lymphatic organs.

In 1908 Rous, using an improved technique, in the collection of samples from thoracic duct fistulæ showed that in dogs the thoracic duct furnished the circulating blood more lymphocytes than had been usually supposed. Also, he stated that muscular activity and certain lymphagogues increased the lymphocyte output.

Davis and Carlson in 1909, in studying the leucocytes in the neck lymph, thoracic lymph, and blood of normal dogs, also noticed a decrease in the number of the mononuclear elements of the blood following ligation of the thoracic duct and neck lymphatics. Unfortunately the observations were carried out for only 48 hours following operation and the subsequent course of the lymphocyte content of the blood was not followed; likewise it is uncertain to what extent the operation did prevent the entrance of lymph into the blood stream, since the lymphatics were not subsequently injected.

Bunting and Huston were also interested in the fate of the lymphocyte,—a question which Davis and Carlson had previously considered. Bunting and Huston splenectomized rabbits and found that the number of lymphocytes in the circulating blood increased. About a week following splenectomy the left thoracic duct as well as the neck lymphatics on both sides were ligated, and it was found that the number of lymphocytes in the circulating blood decreased. They also observed lymphatics going from the left thoracic duct through the thymus to the right thoracic duct, and concluded that the lymphocyte count was restored through such channels; however, they did not keep their animals long enough to note the return of the lymphocytes to their preoperative level. The authors advanced the hypothesis that the lymphocytes may migrate for instance into the mucous membrane of the intestine and from there into the lumen of the intestine, since more lymphocytes enter the blood stream *via* the thoracic duct in 24 hours than are present in the blood at any one time.

From the above discussion it will be seen that although all the investigators are impressed with the importance of the thoracic duct as an avenue for the supply of lymphocytes to the circulating blood, yet no one has made a consistent attempt to analyze the changes that take place in the animal economy following ligation of the thoracic duct in order that the normal number of lymphocytes be again restored to the blood stream; nor has any one determined the approximate time following ligation of the duct at which a collateral circulation of the thoracic duct has mediated in the establishment of the normal preligation supply of lymphocytes to the blood stream.

Whether lymphocytes may enter the blood stream directly at their site of formation, as in the lymph glands and spleen, is another factor that enters into the problem. According to von Schumacher, who has studied the lymph glands in this respect, the blood in the vein leaving a lymph gland has more leucocytes than that in a neighboring artery. Unfortunately he did not study the blood in the artery going to the lymph gland in question; also, no figures were given to indicate the extent of the difference in the number of leucocytes in the afferent and efferent blood vessels.

Besides the definite decrease in the number of lymphocytes following ligation there were striking changes in other cell groups. The polymorphonuclear neutrophils invariably increased greatly in number immediately after the operation, but then gradually returned to a normal level. Contrariwise, the eosinophils decreased in number following operation but soon returned to their preligation level. The number of mononuclears and transitionals did not seem to have been influenced much by the operation.

The hematology of the laboratory animals does not seem to have been studied very extensively. Burnett, Busch and Van Bergen, Goodall, and Klieneberger and Carl all give figures for the differential counts of cat blood. Oddly enough the normal differential counts from Cat 1 corresponded to those of Goodall, while the counts from Cat 2 resembled those of Klieneberger and Carl. The latter were unable to find any mast cells in the blood.

SUMMARY.

This report has attempted to analyze the changes in the absolute number of small lymphocytes in the blood stream of the cat following the intrathoracic ligation of the thoracic duct. Such a ligation produced an immediate decrease in the number of small lymphocytes to the extent of 56 per cent, but it was found that the preoperative level was again reached at about the end of 3 weeks. One is led to believe that the gradual return of the number of small lymphocytes to the preligation level took place *pari passu* with the establishment of the collateral circulation of the thoracic duct, although there is no absolute proof of this. Yet it is definite that the thoracic duct is an important avenue for the entrance of small lymphocytes into the blood

stream, and that it is the pathway through which at least half of the small lymphocytes reach the circulating blood in the cat.

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